Exercise and the Gut Microbiome: A Review of the Evidence, Potential Mechanisms, and Implications for Human Health

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MAILING, L.J., J.M. ALLEN, T.W. BUFORD, C.J. FIELDS, and J.A. WOODS. Exercise and the gut microbiome: a review of the evidence, potential mechanisms, and implications for human health. *Exerc. Sport Sci. Rev.*, Vol. 47, No. 2, pp. 75–85, 2019. *The gastrointestinal tract contains trillions of microbes (collectively known as the gut microbiota) that play essential roles in host physiology and health. Studies from our group and others have demonstrated that exercise independently alters the composition and functional capacity of the gut microbiota. Here, we review what is known about the gut microbiota, how it is studied, and how it is influenced by exercise training and discuss the potential mechanisms and implications for human health and disease.* **Key Words:** endurance exercise, gut microbiota, short-chain fatty acids, butyrate, gut health, inflammatory bowel disease

Key Points

- The trillions of microbes in the gut play essential roles in human health.
- Exercise training alters the composition and functional capacity of the gut microbiota, independent of diet.
- Exercise-induced alterations of the gut microbiota may depend on obesity status, exercise modality, and exercise intensity.
- Exercise-induced alterations of the gut microbiota are likely to have numerous benefits for human health.

INTRODUCTION

Scientists only recently have begun to appreciate the human gut as a complex ecosystem of bacteria, archaea, eukaryotes, and viruses that have co-evolved with humans over thousands of years. Known collectively as the gut microbiota, these microbes can weigh up to 2 kg and are imperative to host digestion, metabolic function, and resistance to infection (1). The human gut microbiota has an enormous metabolic capacity, with over 1000 different unique bacterial species and over 3 million unique genes (2). Collectively, the sum of the microbial genes in the gut is called the gut microbiome.

Given the numerous roles of the gut microbiota in host physiology and pathophysiology, it is not surprising that there is great interest in identifying ways to manipulate microbial communities in health and disease (3–5). Although diet is well known to modulate the composition of the gut microbiota, recent studies suggest that exercise can alter gut microbial communities as well. This will be the focus of the present review. Key questions include the following: does exercise independently alter the gut microbiota? If yes, by what mechanism? With what implications for the gut and other organ systems? Can exercise beneficially modulate the gut microbiota in states of disease? Before we attempt to answer these questions, we will first review advances in technology that have improved the understanding of the microbiota's contribution to health and disease and enabled investigations into exercise's impact on gut microbial communities.

BASIC MICROBIOME METHODOLOGY

Until the 1990s, scientific study of gut microbes primarily relied on culture, staining, and microscopy (6). Growth media and conditions typically favored fast-growing, aerobic microbes, meaning that many anaerobic microbes could not be effectively cultured or studied (7). This changed with the advent of DNA sequencing. 16S bacterial ribosomal RNA (rRNA) gene sequencing (hereafter 16S) quickly became the most popular (8). The conserved regions of this gene are used to design broad-spectrum polymerase chain

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reaction (PCR) primers that allow for the amplification of the more rapidly evolving hypervariable regions across a broad spectrum of microbes (Fig. 1). The resulting amplified hypervariable region sequences can then be classified taxonomically by comparing them to a curated database of fully sequenced bacterial 16S genes (9).

16S is still the most widely used method to cost-effectively characterize bacterial communities in a research setting (10), but it does have several limitations. First, taxonomic classifications are limited primarily to bacteria. Second, sequence classification is normally limited to the genus level, as multiple species may have the same sequence within the studied hypervariable region (11). 16S also is susceptible to primer bias (12,13). Finally, 16S analysis does not provide direct information about the function of gut microbes or the potential interactions with host physiology, though tools such as PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) can infer potential functional pathways from 16S results using information from the Human Microbiome Project (14).

TARGETED GENOMICS, META-OMICS, AND METABOLITES

Recently, interest has increased for moving beyond 16S ("who's there?") to better understand the role of the gut microbiota in states of health and disease ("what are they doing?"). One way to do this involves using specific degenerate primers to perform quantitative PCR for a specific conserved microbial gene. For example, our lab has targeted the butyryl-CoA:acetate-CoA transferase gene, which encodes the primary enzyme involved in gut bacterial production of the short-chain fatty acid (SCFA) butyrate (15). This targeted genomics approach is relatively quick and inexpensive but limited in overall scope.

In contrast, metagenomics (*i.e.*, shotgun sequencing) involves assessing the entire gene content of a given microbial community (16) to assess microbial functions and allow for identification of bacteria, archaea, viruses, and fungi with greater specificity (2,17). Current limitations for metagenomics include high cost, sequencing biases, complexity in both data processing and analysis, and incomplete databases for taxonomy and genomic assignment (16). Nevertheless, newer sequencing technologies as well as upgraded downstream pipelines (18,19) and reference databases (20,21) have improved taxonomic and functional genomic profiling of the gut microbiota. Other meta-omics, such as metatranscriptomics, metaproteomics, and metametabolomics, can help elucidate which genes are actually expressed and become functional proteins capable of carrying out diverse metabolic functions (22). These high-throughput, high-resolution techniques are rapidly improving in speed, quality, and cost and will soon be the norm for microbiome research.

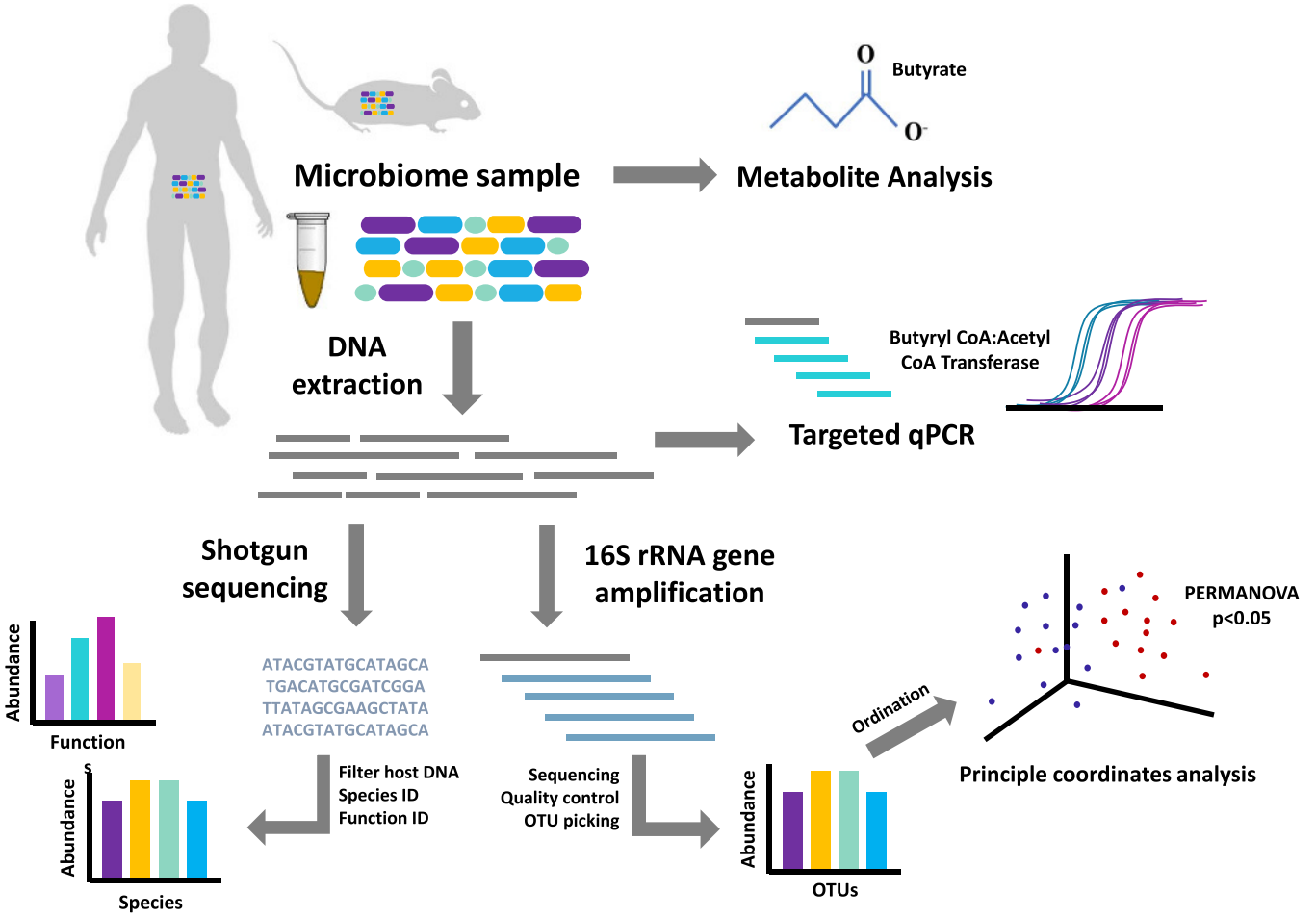

Figure 1. Methodologies commonly used to study the gut microbiome. Fecal or gastrointestinal (GI) content samples can be used for metabolite analysis or undergo chemical and mechanical digestion to extract nucleic acids. Extracted DNA can be used for targeted genomics, shotgun sequencing, or 16S ribosomal RNA (rRNA) gene amplification.

Metabolites also can be measured directly using gas or liquid chromatography and mass spectrometry to provide insight into the collective metabolic function of the gut. However, quantitation of certain volatile metabolites requires prompt collection and acidification or ethanol treatment of samples after collection (23). Moreover, the fecal concentration of many gut metabolites will depend on gut transit time, cross-feeding interactions between microbes, and rate of host absorption (24), so it is not necessarily representative of luminal concentrations.

STATISTICAL INTERPRETATION OF GUT MICROBIOME DATA

Microbiome data analysis typically is performed using an open-source bioinformatics pipeline, such as Quantitative Insights Into Microbial Ecology 2 (QIIME 2) (25) or Mothur (26) for 16S and Metagenomics Reports (METAREP) (27), Metagenome Analyzer (MEGAN) (28), or Metagenomic Rapid Annotations using Subsystems Technology (MG-RAST) (29) for comparative metagenomics. For 16S, sequences from samples must be demultiplexed, quality filtered, and clustered into operational taxonomic units (OTUs) based on sequence identity (ID) (*i.e.*, about 95% ID for genus; 97% ID for species). These are then taxonomically classified using common reference databases (30,31) and visualized as a phylogenetic tree or represented using bar plots.

The alpha (α)-diversity metrics Chao1, Shannon index, and Simpson index are a measure of the diversity *within* a sample and take into account both the number of unique OTUs in a sample (richness) and the relative abundance of these OTUs (evenness). In contrast, beta (β)-diversity metrics like Bray-Curtis and UniFrac are measures of the diversity *between* samples. When more than two samples are used, β-diversity is calculated for every pair of samples to create a distance (dissimilarity) matrix. The data present in the β-diversity distance matrix can be visualized using a 2-D or 3-D Principle Coordinates Analysis plot, where each axis explains a certain percentage of variation present in the dataset. Each sample is represented by a single point, and the distance between points reflects how compositionally different the samples are from one another (32). Statistical significance of community-level differences can be assessed using a PERMANOVA test (33).

FACTORS INFLUENCING THE GUT MICROBIOME

Using these methodologies, several factors have been identified that influence gut microbial composition and metabolic capacity beginning at birth. The fetal gut contains few if any microbes as the womb is largely sterile (34). Microbial colonization begins at birth and is significantly influenced by mode of delivery (vaginal or Cesarean section) and infant diet (breastmilk or formula) (35). Other factors, including increased sanitation, reduced exposure to infection through vaccination, elimination of enteropathogens, and exposure to antibiotics and nonantibiotic drugs also can alter the commensal, or native, microbiota (36). Dietary intake also has a significant impact on microbial composition throughout life (37).

EXERCISE AND THE GUT MICROBIOME: EVIDENCE IN ANIMAL STUDIES

Emerging research from our group and others suggests that exercise also influences the gut microbiota. Over a dozen controlled animal studies have shown that exercise training independently alters the composition and functional capacity of the gut microbiota (38–51). Matsumoto *et al.* (46) was the first to find that 5 wk of exercise training resulted in an increase in the bacterial metabolite butyrate. Several other studies have recapitulated this finding and shown that exercise training increases the relative abundance of butyrate-producing taxa (38,41). Butyrate is an SCFA produced from the bacterial fermentation of dietary fiber. As the primary fuel for colonocytes, butyrate has been shown to increase colonic epithelial cell proliferation, promote gut barrier integrity, and regulate the host immune system and gene expression (52,53).

Drawing other broad conclusions as to how and to what degree exercise alters the rodent gut microbiota has proved difficult because of incongruities in diet, species/strain, animal age, and exercise modality used. For instance, several studies suggest that exercise increases the ratio of Firmicutes to Bacteroidetes phyla (38–40), whereas some studies suggest that exercise reduces this ratio (41–44). Still, others have found no change at the phylum level (45,46). Several factors may influence the disparate results observed in these studies. For instance, we recently reported that voluntary wheel running (VWR) and forced treadmill running (FTR) — the two most common modes of endurance exercise used with rodents — differentially altered the gut microbiota (47). Mika *et al.* (44) found that microbial genera were more robustly altered by VWR in juvenile, compared with the adult rats, whereas Evans *et al.* (41) found that VWR increased microbial diversity, but only in mice fed a high-fat diet.

EXERCISE AND THE GUT MICROBIOME: HUMAN CROSS-SECTIONAL EVIDENCE

Evidence for a role of exercise in shaping the human gut microbiota first emerged from cross-sectional studies (Table). Clarke *et al.* (54) found that the gut microbiota of professional rugby players had greater alpha diversity and a higher relative abundance of 40 different bacterial taxa than the gut microbiota of lean sedentary controls. The athletes also had lower abundance of *Bacteroides* and *Lactobacillus* species than their lean sedentary counterparts (54). More recently, Bressa *et al.* (55) compared active women with sedentary controls and observed that women who performed at least 3 h of exercise per week had increased levels of *Faecalibacterium prausnitzii*, *Roseburia hominis*, and *Akkermansia muciniphila*. *F. prausnitzii* and *R. hominis* are known butyrate producers (56), whereas *A. muciniphila* has been associated with a lean body mass index (BMI) and improved metabolic health (57).

Several studies also have attempted to correlate the composition and metabolic capacity of the microbiota with cardiorespiratory fitness. Durk *et al.* (58) showed that a higher ratio of Firmicutes to Bacteroidetes, the two predominant phyla in the human gut microbiota, was significantly correlated with maximal oxygen uptake ($\dot{V}O_{2max}$). Estaki *et al.* (59) found that in younger adults, microbial diversity and abundance of butyrate-producing bacterial taxa were positively correlated with cardiorespiratory fitness, whereas Barton *et al.* (60) showed, using metagenomic analyses, that athletes have altered gut microbial pathways for amino acid biosynthesis and carbohydrate metabolism and greater fecal SCFA concentrations.

Nevertheless, all of these studies were limited by their cross-sectional design and their inability to control for the effects of diet (and perhaps other factors) on the gut microbiota. There

TABLE. Summary of cross-sectional and longitudinal studies assessing the impact of physical activity status or an exercise intervention on the human gut microbiome

Study	Design	Subjects	Exercise Training	Change or Control of Diet	Impact on Gut Microbial Communities
Clarke *et al.*, (54)	Cross-sectional	Elite rugby players (n = 40), low BMI controls (n = 23), and high BMI controls (n = 23)	n/a	Elite athletes consumed significantly more protein and total energy. Increased protein intake accounted for many observed differences in gut microbial composition	Greater alpha diversity in elite athletes compared with lean sedentary controls. ↑*Akkermansia* in athletes and low BMI controls; ↑Erysipelotrichaceae, S24-7, Prevotella, and Succinivibrio and ↓Lactobacillaceae, Bacteroides, and *Lactobacillus* species in athletes compared with lean controls
Estaki *et al.*, (59)	Cross-sectional	Healthy adults with varying cardiorespiratory fitness levels (n = 39)	n/a	Protein intake was highly associated with overall microbial community composition	$\dot{V}O_{2peak}$ accounted for more than 20% of the variation in species richness. Individuals with higher fitness had increased relative abundance of butyrate-producing taxa and increased fecal butyrate concentrations
Stewart *et al.*, (110)	Cross-sectional	Adult males type 1 diabetics with good glycemic control and high levels of physical fitness (n = 10) and matched healthy adult male controls (n = 10)	n/a	Not assessed	Gut microbial composition of patients with type 1 diabetes in good glycemic control and with high physical fitness levels is comparable to those of matched people without diabetes
Bressa *et al.*, (55)	Cross-sectional	Premenopausal women, active (>3 h of physical exercise/wk, n = 19) or sedentary (<30 min 3 d·wk^{-1}, n = 21)	n/a	Greater consumption of fruits and vegetables by active group; sedentary group ingested more processed meats	Increased relative abundance of *F. prausnitzii*, *R. hominis*, and *A. muciniphila* in active women; reduced relative abundance of Barnesiellaceae and Odoribacteraceae
Yang *et al.*, (111)	Cross-sectional	Premenopausal women, all activity levels, primarily overweight or obese (n = 71)	n/a	No significant differences between groups in macronutrient composition, fiber, or total energy intake	Lower $\dot{V}O_{2max}$ was associated with lower relative abundance of *Bacteroides* species and higher relative abundance of *Eubacterium rectale–Clostridium coccoides* group
Barton *et al.*, (60)	Cross-sectional	Professional rugby players (n = 40) and sedentary controls with low BMI (n = 22) or high BMI (n = 24)	n/a	Rugby players consumed significantly more protein and total energy	Rugby players had increased amino acid and antibiotic biosynthesis, carbohydrate metabolism, and increased fecal SCFAs compared with controls
Durk *et al.*, (58)	Cross-sectional	Healthy young adults (n = 20 males, n = 17 females) with varying cardiorespiratory fitness level	n/a	No association between macronutrient intake and Firmicutes to Bacteroidetes ratio	Higher ratio of Firmicutes to Bacteroidetes was significantly correlated with $\dot{V}O_{2max}$. $\dot{V}O_{2max}$ accounted for 22% of the variance in gut microbiota composition
Paulsen *et al.*, (112)	Longitudinal	Post-primary treatment breast cancer survivors (n = 12)	Received written materials regarding benefits of physical activity. Fitness measured at baseline and 3 months	No significant changes in self-reported carbohydrate or fiber intake. Other macronutrients not reported or controlled for	Significant association between cardiorespiratory fitness and beta diversity at 3-month timepoint
Allen *et al.*, (61)	Longitudinal	Previously sedentary lean or obese adults (n = 32)	6-wk progressive aerobic exercise intervention (moderate-high intensity) + 6-wk sedentary washout period	Diet stability confirmed using 7-d diet diaries and a 3-d control diet before each fecal collection	Several taxa were differentially altered depending on BMI status: *Faecalibacterium* increased in lean subjects but decreased in obese; *Bacteroides* decreased in lean subjects but increased in obese. Increased butyrate-producing taxa, fecal acetate, and butyrate concentrations. Effects were reversed upon return to sedentary lifestyle
Cronin *et al.*, (62)	Longitudinal	Predominantly overweight or obese adults randomized to exercise-only (E), exercise + whey protein (EP), or whey protein only (P) groups (n = 30 each group)	8-wk mixed progressive moderate aerobic exercise (18–32 min) and resistance training (3×/wk)	Self-reported maintenance of dietary intake	No significant changes in taxonomic composition; trend for increase in bacterial diversity in E and EP groups. Only modest alterations of microbial metabolic potential
Munukka *et al.*, (63)	Longitudinal	Previously sedentary, overweight women (n = 17)	6-wk cycling exercise (low-moderate intensity)	Diet stability confirmed in 14 subjects using 3-d food record; only slight increase in energy derived from starch	Increased relative abundance of *Akkermansia* and decreased relative abundance of Proteobacteria. Only half of the subjects' microbiomes responded to exercise. Exercise training decreased abundance of fructose and amino acid metabolism-related genes

is considerable inter-individual variability in the composition of the microbiota, and active individuals tend to eat differently from sedentary individuals. For instance, Clarke *et al.* (54) found that increased protein intake by elite rugby players accounted for many of the observed differences in the gut microbiota. These limitations suggested the need for longitudinal studies to determine whether exercise independently alters the gut microbiota in humans.

EXERCISE AND THE GUT MICROBIOME: HUMAN LONGITUDINAL STUDIES

Recently, members of our group published findings from the first controlled longitudinal study to assess the effects of exercise on the gut microbiome (61). In total, 32 sedentary adults (lean [BMI, <25] or obese [BMI, >30]) participated in a 6-wk supervised endurance exercise program (30- to 60-min duration, 3× per week) with stringent dietary controls. Several taxa were differentially

altered by exercise depending on BMI status. For instance, exercise increased *Faecalibacterium* species in lean subjects but reduced its abundance in obese subjects; *Bacteroides* species decreased in the lean subjects and increased in the obese subjects. Six weeks of exercise also increased the abundance of butyrate-producing taxa and fecal acetate and butyrate concentrations, but only in lean subjects. Interestingly, most bacterial taxa and SCFAs that increased with exercise subsequently decreased during the 6-wk sedentary washout period that followed, indicating that the effects of exercise on the microbiota were both transient and reversible.

Similarly, Cronin *et al.* (62) sought to determine whether a short-term exercise regime, with or without whey protein supplementation, could alter gut microbial composition and function in predominantly overweight or obese male and female adults (n = 90). Those randomized to the exercise groups were required to perform moderate-intensity aerobic training (18- to 32-min duration) and a progressive resistance training program three times per week for 8 wk. Post-intervention assessment did not reveal any significant changes in taxonomic composition or metabolic pathways in either exercise group compared with baseline. However, a trend was seen for an increase in bacterial diversity in the exercise and exercise + whey protein groups, compared with the group that received whey protein alone. Metagenomic and metabolomic analyses revealed only modest alterations of microbial metabolism. Although the study had a fairly large sample size, the authors note that self-reported maintenance of usual dietary intake and a wide BMI range may have prevented detection of more significant changes.

Munukka *et al.* (63) performed a similar study to determine whether endurance exercise could affect the gut metagenome in previously sedentary overweight women (n = 17). Six weeks of light- to moderate-intensity cycling resulted in an increased relative abundance of *A. muciniphila* and a decrease in Proteobacteria. Most interestingly, only about half of the subjects' microbiomes responded considerably to exercise. Metagenomic analysis revealed that exercise training decreased the abundance of several genes related to fructose and amino acid metabolism.

Together, these findings suggest that exercise has independent effects on the gut microbiota, but longer duration or higher intensity aerobic training may be required to induce significant taxonomic and metagenomic changes. Furthermore, the microbiota of lean individuals may be more responsive to an exercise intervention than that of overweight or obese individuals.

POTENTIAL MECHANISMS

There are several potential mechanisms by which exercise might alter the gut microbiota (Fig. 2). The gut-associated lymphoid tissue, or GALT, lies through the small and large intestine and contains about 70% of the body's immune cells. Several animal studies performed by Hoffman-Goetz *et al.* (64–66) have found that exercise alters the gene expression of intraepithelial lymphocytes, downregulating pro-inflammatory cytokines and upregulating anti-inflammatory cytokines and antioxidant enzymes. These immune cells reside in close proximity to microbial communities and produce antimicrobial factors that are essential for mediating host-microbial homeostasis (67).

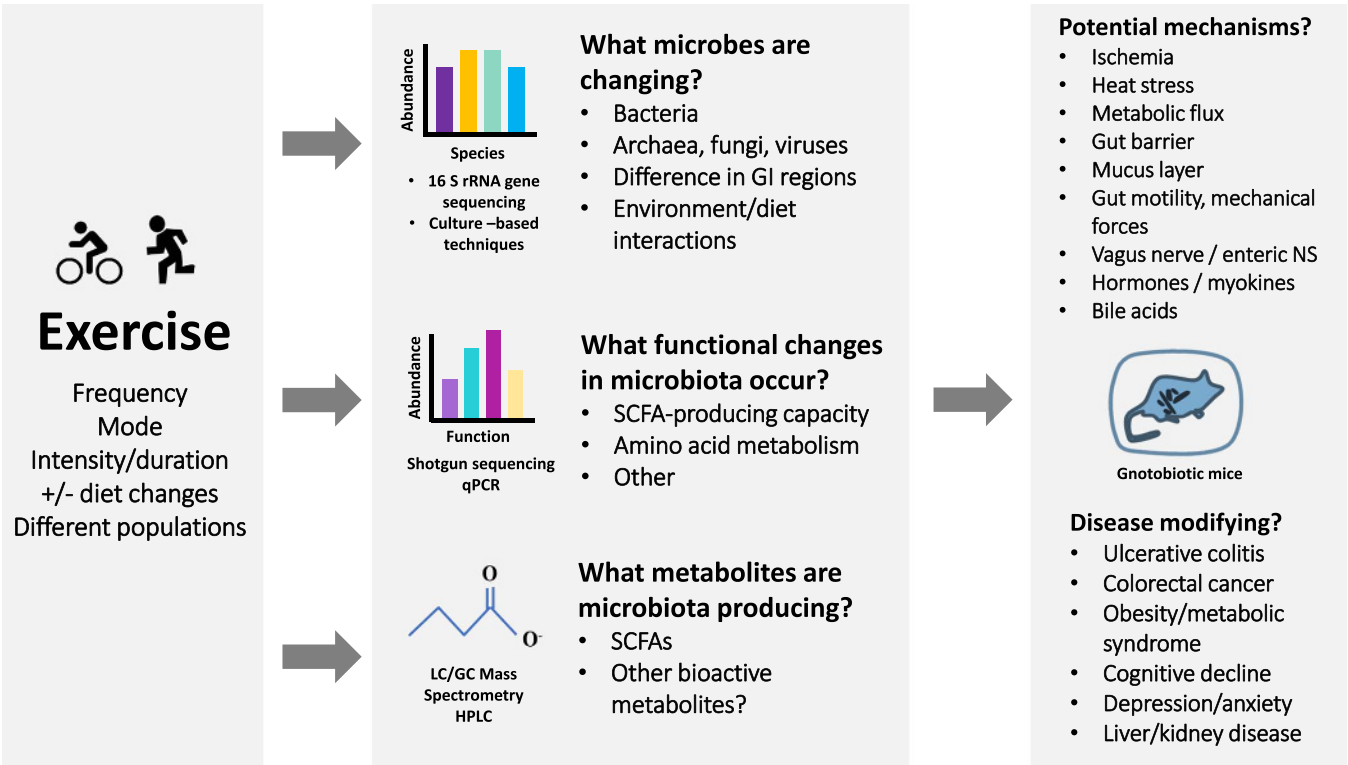

Figure 2. Current unknowns and future areas of research related to exercise and the gut microbiome. Although several studies have now shown that exercise alters gut microbiota composition, functional capacity, and metabolites, the effects of different exercise frequencies, modes, and intensities are unknown. Assessing the effects of exercise on the gut microbiota in different populations and its synergy with different diets also represents a key area of future research. Mechanistic studies, such as those that use mice, will help determine the potential mechanisms involved, and whether exercise-induced changes in the gut environment are potentially disease modifying. GI, gastrointestinal; NS, nervous system; SCFA, short-chain fatty acid.

Similarly, exercise may impact the integrity of the gut mucus layer, which plays an important role in keeping microbes from adhering to the gut epithelium and serves as an important substrate for certain mucosa-associated bacteria, such as *A. muciniphila*.

Exercise raises core temperature and results in heat stress, particularly when performed for long durations or in a hot environment (68). Exercise also can reduce intestinal blood flow by more than 50%, with significant gut ischemia occurring within 10 min of high-intensity exercise (69). Upon rest, the splanchnic bed undergoes rapid reperfusion. Although the intestine is an anaerobic environment, gut epithelial cells primarily use oxidative metabolism, and high-intensity exercise is known to transiently impair gut barrier function (69,70). Thus, exercise-induced heat stress and ischemia may briefly result in more direct contact between the gut mucosal immune system and the microbes that reside in the gut lumen and mucosa, with potential consequences for gut microbial communities.

Although intestinal permeability occurs briefly during acute exercise, contact between microbes and the immune system may be reduced at rest with regular physical activity. Trained athletes have lower levels of circulating bacterial endotoxin lipopolysaccharide at rest than sedentary individuals (71) and a greater heat shock protein response to heat stress (72). Increased heat shock proteins in the gut have been shown to prevent breakdown of tight junction proteins between epithelial cells (73). Thus, it is plausible that exercise represents a hormetic stressor to the gut that stimulates beneficial adaptations and improves the long-term resilience of the gut barrier.

Altered gut motility or activity of the enteric nervous system is another mechanism by which exercise may influence the gut microbiome. Exercise reduces transit time in the large intestine and has been shown to accelerate the movement of gas through the gastrointestinal (GI) tract (74,75). Exercise also is well known to impact the autonomic nervous system increasing vagal and overall sympathetic tone (76), but its impact on the complex mesh-like network of neurons that innervate the gut has not been well elucidated. Nonetheless, regional or global changes in GI transit are likely to have profound effects on intestinal pH, mucus secretion, biofilm formation, and availability of nutrients to microbes. Mechanical forces also are increased in the abdomen during most forms of aerobic exercise, which could potentially influence gut motility or increase the mixing of intestinal contents.

Exercise training also may alter the enterohepatic circulation of bile acids. Meissner *et al.* (77) found that hypercholesterolemic mice that were given access to a running wheel for 12 wk displayed increased bile acid secretion and increased fecal bile acid outputs compared with hypercholesterolemic mice that remained sedentary. Bile acids are potent regulators of gut microbiota community structure, and an absence of these molecules is associated with significant alterations in gut microbial communities (*i.e.*, gut dysbiosis) (78). Thus, changes in the bile acid pool could significantly shift the gut microbiome with exercise.

Lastly, exercise significantly alters metabolic flux (the rate of turnover of molecules through metabolic pathways) and requires contraction of skeletal muscle, which stimulates the release of myokines, metabolites, and neuroendocrine hormones that may interact with the gut directly or indirectly through a common interface with the immune system (79). Significant amounts of lactate are released into the blood during exercise, which could alter intestinal pH if any of this lactate is secreted into the gut lumen. Overall, more research is needed to determine which of these mechanisms are responsible for the adaptation of the gut microbiota to exercise training.

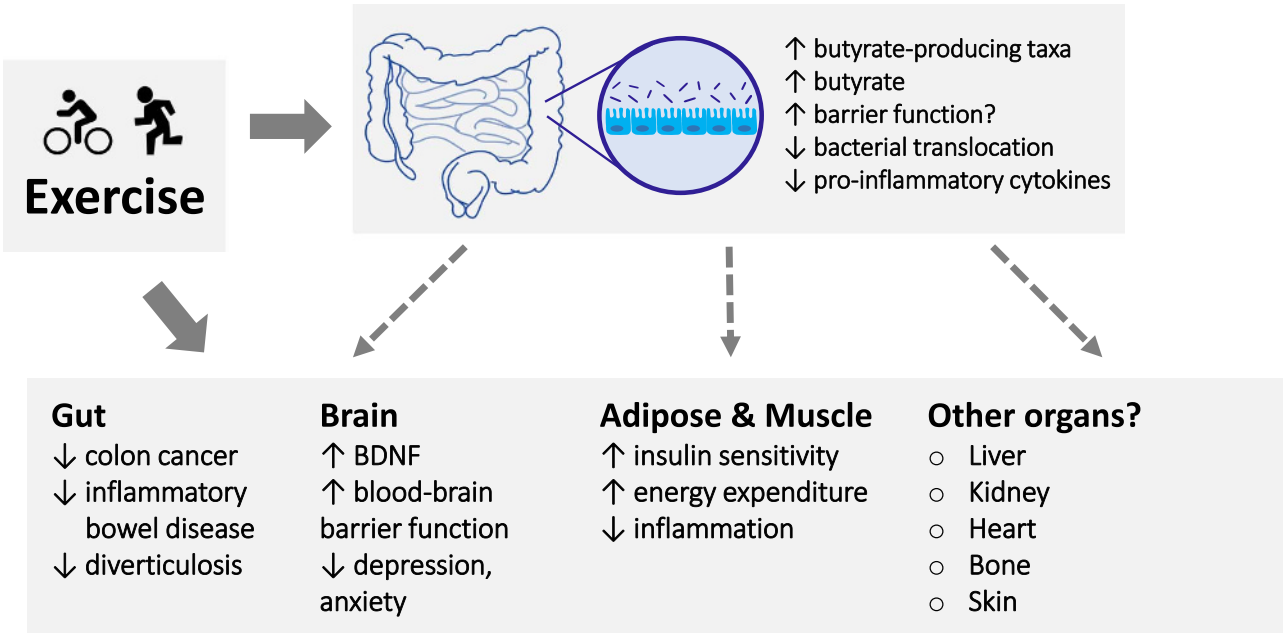

Figure 3. Proposed model for how exercise alters the gut microbiota and gut epithelium with potential implications for human health. Exercise has been shown to increase butyrate-producing taxa and fecal butyrate concentrations and reduce pro-inflammatory cytokines and oxidative stress in the gut. Exercise also is known to have benefits on whole-body physiology and is protective against colon cancer, inflammatory bowel disease (IBD), depression, anxiety, and obesity. Whether this disease protection is mediated by exercise-induced changes in the gut microbiome and gut epithelium remains to be determined. BDNF, brain-derived neurotrophic factor.

IMPLICATIONS FOR THE GUT AND BEYOND

Exercise-induced alterations of the gut microbiota likely have implications for gut and whole-body health. Physical activity has been shown to be protective against many chronic diseases and offers an attractive and cost-effective way to improve quality of life (80). Though under-recognized to date, many of these benefits may be derived via interactions with the gut microbiota (Fig. 3). Here, we discuss potential example conditions for which the gut microbiota may play a pivotal role, though they almost certainly do not represent the full spectrum of potential benefits. It also should be noted that in most cases, the potential attribution of beneficial effects to the gut microbiota remains speculative because of the lack of definitive data in this area.

Colorectal Cancer

Observational studies indicate that physically active individuals have a 24% reduced risk for colorectal cancer compared with sedentary individuals (23,81). Beginning an exercise program after the onset of colorectal cancer also may improve quality of life and reduce overall mortality (81). In preclinical animal studies, VWR has been shown to reduce colon tumor incidence (82). One mechanism for this may be increased butyrate production from exercise. Colorectal cancer patients have been shown to have an altered gut microbiota characterized by a reduced abundance of butyrate-producing taxa, including *Roseburia* and *Lachnospiraceae* (83).

In vitro studies have shown that butyrate differentially regulates gene expression in healthy and cancerous cells (84). In healthy epithelial cells, butyrate is rapidly metabolized via the mitochondrial tricarboxylic acid cycle. This results in a buildup of cytosolic citrate and acetyl CoA and increases the acetylation of histones by histone acetyltransferases. This epigenetic modification increases expression of genes involved in cell proliferation and cell turnover, effectively strengthening the intestinal barrier (84).

In colorectal cancer cells, however, mitochondrial dysfunction results in an accumulation of butyrate in the cytosol. Free butyrate inhibits histone deacetylases, which results in the epigenetic suppression of proliferation and promotion of cell death pathways (84). This may ultimately lead to a reduction in tumor size and reduces the chance of metastasis. Indeed, Basterfield and Mathers (85) found that Min mice, which are genetically predisposed to intestinal adenomas, had a reduced number of large tumors in the colon and a trend toward reduced tumor multiplicity with exercise training. There was a weak correlation between fecal butyrate concentrations and tumor number (85).

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) includes both Crohn's disease and ulcerative colitis (UC) and is characterized by inappropriate gut immune responses and an altered microbiota. IBD patients have an increased relative abundance of Enterobacteriaceae and reduced abundance of *Roseburia*, a genus known to produce butyrate and induce regulatory T cell formation (86). Regulatory T cells are important for modulating the immune system, promoting tolerance to self-antigens, preventing autoimmune disease, and dampening inflammation. Metagenomic analysis also revealed reduced carbohydrate metabolism and amino acid biosynthesis in the fecal microbiome of IBD patients compared with healthy controls (86) — two pathways that exercise has been reported to increase. Indeed, higher self-reported physical activity levels are associated with a 22% reduced risk of active UC (87), and a 10-wk intervention that included moderate exercise improved quality of life in patients with moderately active UC (88).

Our group and others have performed several preclinical animal studies on the effects of exercise on colitis. Szalai *et al.* (89) found that 6 wk of VWR increased expression of heme oxygenase and nitric oxide synthase, increased anti-inflammatory cytokines, and reduced inflammatory markers and the severity of mucosal damage in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, whereas Liu *et al.* (90) found that 1 month of VWR suppresses pro-inflammatory cytokine production in response to dextran sodium sulfate (DSS)-induced colitis by up-regulating glucocorticoid-mediated peroxisome proliferator-activated receptor gamma (PPAR-γ) expression in the colon. PPAR-γ regulates fatty acid storage and glucose metabolism. In 2013, members of our group confirmed that VWR conferred protection against DSS-induced colitis and reduced disease-related symptoms and mortality, but additionally observed that FTR exacerbated symptoms and led to higher mortality (91). Further study revealed that VWR and FTR resulted in distinct changes in the gut microbiota (47).

To determine if exercise-induced alterations in the gut microbiota were directly responsible for the protective effects of VWR, members of our group transferred cecal contents from exercised or sedentary mice into naive, sedentary germ-free mice in the first-ever "exercise" fecal microbiota transplant (FMT). When recipient mice were later subjected to an acute colitis insult with DSS, those that had received a microbiota from exercised mice lost significantly less body weight and had fewer clinical symptoms than those that received a microbiota from sedentary mice. Mice receiving the microbiota from exercised mice also had a more regenerative cytokine profile, with significantly higher levels of transforming growth factor beta (TGF-β), forkhead box P3 (FoxP3), and interleukin (IL-22) gene expression in the distal colon (92). More studies are needed to determine whether exercise can beneficially modulate the gut microbiota in humans with IBD, and whether compositional alterations parallel improvements in symptomology.

Obesity and Metabolic Disease

Several studies have shown that the gut microbiota is closely associated with obesity and metabolic syndrome. A seminal paper by Turnbaugh *et al.* (93) showed that transplanting fecal material from an obese mouse into a germ-free mouse resulted in rapid weight gain. The obese microbiota has a significantly higher capacity for energy harvest from the diet and also may promote intestinal permeability, allowing the influx of endotoxin into the bloodstream. Endotoxemia itself has been shown to result in weight gain and insulin resistance (94).

Evidence from animal studies suggests that exercise may attenuate the gut dysbiosis and altered intestinal villi morphology induced from high-fat diet feeding (45). Queipo-Ortuño *et al.* (42) found that just 6 d of VWR increased the relative abundance of *Lactobacillus* and *Bifidobacterium* species in male rats, which were positively correlated with serum leptin levels, whereas Lambert *et al.* (40) found significant interactions between exercise and diabetic state on the gut microbiota in an animal model of type 2 diabetes.

Lai *et al.* (95) showed that high-fat diet–fed obese mice receiving FMT from exercised, normal-fat diet–fed donor mice

showed improvements in metabolic parameters, including weight loss, reduced fasting blood glucose, and lower hepatic expression of pro-inflammatory cytokines. Notably, two of the taxa that were highly associated with FMT from exercised donors, *Odoribacter* and AF12 of the family Rikenellaceae, are known butyrate producers. In animal models of obesity, butyrate has been shown to increase energy expenditure, improve insulin sensitivity, and reduce adiposity (96). Butyrate and other SCFAs also stimulate the production of satiety hormones, which help regulate food intake, and may help delay or attenuate the development of diabetes by improving gut barrier function (97).

Mental and Cognitive Health

The gut microbiota also has been implicated in mental health and cognition, and the existence of a gut-brain axis is well established (98,99). Gut microbiota–derived metabolites have been shown to activate receptors on vagal afferents of the enteric nervous system, and certain microbes also are capable of producing neurotransmitters; for example, *Lactobacillus* species can produce both serotonin and gamma-aminobutyric acid (GABA) (100). Serotonin is thought to play a role in emotion and cognitive functions, and low levels have been linked to depression. GABA is the chief inhibitory neurotransmitter in the central nervous system and typically has anti-anxiety and relaxant effects. Thus, it is no surprise that germ-free mice that lack a commensal microbiota exhibit altered brain function, abnormal behaviors, and an exaggerated hypothalamic-pituitary-adrenal response to stress (101).

Gut dysbiosis also may contribute to impaired mental health. Human patients with major depressive disorder have an altered gut microbiota, characterized by changes in the relative abundance of Firmicutes, Bacteroidetes, and Actinobacteria (102). Notably, transferring fecal material from these patients into germ-free mice confers depression-like behaviors in the recipient mice (102). Stevens *et al.* (103) found that patients with a depressive or anxiety disorder had a unique predicted gut metagenomic profile and increased levels of plasma markers of intestinal permeability.

Exercise is well known to have benefits for mental and neurological health (104), and it is plausible that some of the beneficial effects of exercise on the brain are mediated by the gut microbiota. For instance, Kang *et al.* (38) found that an hour of daily wheel running increased the relative abundance of Lachnospiraceae, a family of known butyrate-producing microbes, which was negatively correlated with anxiety-like behavior in adult C57Bl/6J mice. Butyrate itself has been shown to upregulate brain-derived neurotrophic factor expression in the hippocampus and frontal cortex of mice, which helps to support the survival of existing neurons and encourage the formation of new neurons and synapses. Butyrate also has been shown to regulate the activation of microglial cells, a specialized population of immune cells in the brain (105,106). Like exercise, butyrate also seems to increase neuroplasticity and has anti-depressant activity, boosting brain serotonin levels (107).

FUTURE PERSPECTIVES

Overall, increasing evidence suggests that regular aerobic exercise confers benefits to the gut microbiota, which may be partially responsible for the widespread benefits of regular physical activity on human health. This area of research will no doubt have many exciting developments in the coming decade, and there are many questions that are yet to be answered (Fig. 2). In addition to elucidating the mechanisms involved, the effects of different forms of exercise necessitate further study. Open questions include the following: "What frequency, mode, or intensity of exercise is best? How does exercise impact the gut microbiome in children or the elderly? In healthy or diseased states? How does exercise interact with diet in shaping the gut microbiome? Do probiotics or prebiotics influence gut responses to an exercise intervention? What about resistance exercise?"

Future research also should use methodologies to elucidate the effects of exercise on the microbiome in various regions of the GI tract, including microbes associated with the gut mucus layer. Although this will likely involve more invasive endoscopic procedures for human studies, it is critical to understand the true dynamics of the gut environment. A recent study by Zmora *et al.* (109) suggests that fecal samples often under- or over-represent the relative abundance of various bacterial genera and species in the human gut.

Although we have learned a great deal about how exercise influences bacterial communities, future research also should seek to understand how exercise influences archaea, fungi, and viruses in the human gut and how exercise influences gut competition and ecological patterns. The increasing feasibility of metagenomic studies also will help to elucidate which bioactive metabolites produced by the gut microbiota might be most affected by exercise training. Gnotobiotic, or germ-free, animal studies also will help to determine how exercise-induced alterations in the gut microbiota are causally linked to alterations in disease risk. Ultimately, we can imagine a future of personalized microbiome-based lifestyle medicine, where baseline gut microbiota, diet, and other host factors might help predict which exercise program might be most effective for a given individual.

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