Exercise and gut immune function: evidence of alterations in colon immune cell homeostasis and microbiome characteristics with exercise training

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There is robust evidence that habitual physical activity is anti-inflammatory and protective against developing chronic inflammatory disease. Much less is known about the effects of habitual moderate exercise in the gut, the compartment that has the greatest immunological responsibility and interactions with the intestinal microbiota. The link between the two has become evident, as recent studies have linked intestinal dysbiosis, or the disproportionate balance of beneficial to pathogenic microbes, with increased inflammatory disease susceptibility. Limited animal and human research findings imply that exercise may have a beneficial role in preventing and ameliorating such diseases by having an effect on gut immune function and, recently, microbiome characteristics. Emerging data from our laboratory show that different forms of exercise training differentially impact the severity of intestinal inflammation during an inflammatory insult (for example, ulcerative colitis) and may be jointly related to gut immune cell homeostasis and microbiota–immune interactions. The evidence we review and present will provide data in support of rigorous investigations concerning the effects of habitual exercise on gut health and disease.

INTRODUCTION

Participation in regular physical activity and moderate exercise improves overall immune function and reduces the incidence of inflammatory disease.[1,2] However, translation of the effects regular exercise has in the gut are grossly understudied and often misunderstood. Intense acute and chronic exercise may induce transient gastrointestinal (GI) symptoms, which normally do not cause long-term problems. These effects are most common in elite endurance athletes who experience symptoms that include nausea, diarrhea and GI bleeding related to bowel ischemia, and mechanical or neuroendocrine factors associated with high exercise intensity and long duration.[3,4] This may be a protective mechanism to reduce end-organ damage by prompting the athlete to reduce exercise intensity or duration.[5] However, this is not the case for individuals who participate in regular moderate exercise intensities of shorter durations. In fact, participation in regular moderate physical activity has been considered protective against intestinal inflammatory disease, such as inflammatory bowel disease[6] and colon diseases,[7,8] and does not usually elicit GI disturbances. The specific mechanism(s) underlying the protective effects of moderate exercise are unknown but are thought to involve the anti-inflammatory effects of exercise in the gut and its ability to modulate the gut microbiota.

OVERVIEW OF THE GI IMMUNE SYSTEM

The intestinal immune system has specialized cells and cellular compartments that facilitate immune activities. These specialized tissues consist of the lamina propria, mesenteric lymph nodes and the intestinal epithelial cells themselves. These tissues assist in immune cell migration and harbor innate and adaptive immune cells (dendritic cells, neutrophils, macrophages, B and Th cells, and regulatory T cells (Treg)), which facilitate the maintenance of intestinal immune homeostasis. These cells process antigens of the intestinal lumen and surrounding tissues (that is, dendritic cells), respond to

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tissue damage and translocated bacteria by orchestrating the appropriate immune responses (that is, neutrophils and macrophages) and produce key mucosal antibodies (that is, B-cell production of IgA) to maintain intestinal mucosa homeostasis. During any intestinal insult, increased numbers and activation of immune cells produce the classic pro-inflammatory cytokines interleukin-6 (IL-6), IL-1β and tumor necrosis factor-α, and anti-inflammatory mediators IL-10 and IL-4. Other important cytokines in the gut are IL-23 and IL-17, which are classic pro-inflammatory mediators of mucosal immune function in the gut and have pathogenic roles in gut inflammatory disease.[9]

In addition to the classic cytokine response, chemokines (produced by immune cells and intestinal epithelial cells) mediate inflammation. For example, CCL6, CCL14 and CCL15 are chemotactic for macrophages, neutrophils and T cells that initiate immune cell honing from the circulation to the intestinal tissues. Therefore, a delicate balance of anti- and pro-inflammatory cells and cytokines exists to maintain intestinal homeostasis.

EXERCISE, THE INTESTINAL IMMUNE SYSTEM AND INFLAMMATION

Our lab has published data supporting the concept that moderate exercise improves immune function, is anti-inflammatory and is protective against inflammatory disease.[10–13] In animals, evidence from our laboratory has demonstrated that moderate intensity forced treadmill running (FTR) was protective against influenza infection and skewed the Th1/Th2 balance towards anti-inflammatory Th2 response prompting a reduction in pro-inflammation (tumor necrosis factor-α, IL-1, interferon-γ) and an elevation of anti-inflammatory cytokines (IL-10 and IL-4).[14] We questioned whether the systemic effects exercise has on immune responses translated to the gut and could explain the protective effects regular physical activity on gut health.

Previous evidence from other laboratories also supports the role of exercise in regulating intestinal immune function. Spagnuolo et al.[15] showed that acute exercise increased oxidative stress but was not associated with intestinal inflammation. Notably, Hoffman-Goetz et al.[16] performed early studies evaluating the intestinal immune cell responses to acute and chronic exercise of varying intensities in the absence of disease, in mice. In response to acute FTR, intestinal lymphocyte turnover was increased.[16] Moreover, Hoffman-Goetz et al.[17] established that voluntary wheel training (VWR) elicited increases in antioxidant capacity, anti-inflammatory and pleiotropic cytokine (that is, IL-10 and IL-6, respectively) expression, whereas pro-inflammatory cytokines (for example, tumor necrosis factor-α) were significantly decreased.[18,19] Further, Packer et al.[20] reported that training also improved intestinal immune function in older mice. Together, these studies promote the concept that exercise training is anti-inflammatory in the gut and confirmed the need to characterize the effects of moderate exercise during induced intestinal inflammation.

We found that the exercise paradigm and the psychological context under which the training is performed appear to be very important aspects in the regulation of gut immune function.[21] In our work investigating FTR and VWR on disease activity and severity in a mouse model ulcerative colitis using dextran sulfate sodium (DSS), we demonstrated that FTR exacerbated inflammation, mortality and morbidity when compared with VWR, which reduced inflammation and morbidity. Interestingly, FTR was perceived as a chronic stressor (for example, resulted in adrenal hypertrophy and thymic atrophy), which probably participated in the exacerbated response to DSS colitis, while VWR (which did not affect adrenal or thymic size) was protective.[21] In order to make inroads to explain the exacerbated DSS inflammatory response after FTR, we rationalized that the significant increase in Ccl6 expression in FTR mice (a potential tissue-specific physiologic stress response) may have increased the resident macrophage population in the colon.[21] Therefore, we proceeded with immunohistochemical analysis of cryosectioned distal colon tissue samples and quantified the presence of macrophages in the lamina propria using anti-F4/80 antibodies. On completion of the staining protocol (Abcam, Cambridge, UK) and counterstaining with Mayer's hematoxolin, the cross-sectional area of each slide was calculated and a blinded observer counted F4/80+ cells under bright-field microscopy (×120 magnification). Immunohistochemical analysis of macrophage presence (F4/80+) in FTR and VWR trained mice revealed that macrophage presence was significantly increased as a result of FTR exhibited by significantly greater numbers of macrophages per cross-sectional area (μm2) (sedentary (SED) vs FTR: $t_7 = 3.62$, $^*P = 0.008$; VWR vs FTR: $t_8 = 3.44$, $^*P = 0.009$; Figure 1a). Macrophage presence was not different between SED and VWR mice ($t_7 = 1.239$, $P = 0.255$). Representative images can be found in Figure 1b. These results suggest that FTR-induced increases of macrophages in the lamina propria may be causally related to the exacerbated pro-inflammatory cytokine production and greater symptomology observed in FTR mice on chemical induction of colitis.[21] Interestingly, VWR did not significantly increase Ccl6 expression, whereas Ccl6 was still significantly increased 7 days after the last exercise bout in the FTR group, suggesting that studies are needed to realize the magnitude and duration of a chronic stress response in the gut. Moreover, these findings may have significant clinical relevance in certain populations

(that is, elite endurance athletes or individuals with chronic stress), who suffer with inflammatory GI disorders and who may benefit from new and improved treatment strategies targeting an overpopulation of homeostatic effector immune cells (that is, macrophages) to reduce inflammation. Future studies are necessary to confirm whether Ccl6 expression has a definitive role in this result and whether this phenomenon occurs in humans.

In addition to being a chemoattractant for macrophages, CCL6 also has anti-bacterial functions in the intestinal lumen. Kotarsky et al.[22] showed that CCL6, which is constitutively expressed in the colon and secreted from intestinal epithelial cells, participates in intestinal mucosal immunity by neutralizing microbes through direct binding and removal. Owing to the bacteria neutralizing effects of CCL6, we examined microbiome characteristics focusing on microbes that may be affected by our DSS-induced ulcerative colitis model or exercise stress.

GUT MICROBIOME AND IMMUNE INTERACTION

Any mechanism by which exercise may regulate the GI immune system is incomplete without discussion of the effects of exercise on the gut microbiota. Made up of prokaryotic bacteria, viruses, yeast and helminthes, the microbial communities that reside in the GI tract have remarkable effects on the GI immune system and host physiology. The relationship between the microbes and the GI immune system is perhaps most vividly portrayed in germ-free mice, as a lack of an established microbiota leads to altered immune phenotypes and aberrant responses to immune challenges in these animals.[23] Remarkably, fecal microbial transplants from animal or human donors into germ-free mice results in transferred metabolic,[24] neurological[25] and immunological[26,27] phenotypes from donor to recipient. Despite these noteworthy relationships between the microbiome and host health, an understanding of the microbes, their products and microbe–host interactions is still incomplete. This gap in understanding includes the mechanisms by which behaviors, including exercise and physical activity, may affect the microbiome and associated metabolites.

The gut microbiota, intestinal mucosa and intestinal immune system have a symbiotic relationship under normal, healthy circumstances. More specifically, a normal gut microbiota has an integral role in the development of healthy intestinal mucosal and cellular immunity,[28] whereas dysbiosis can result in the development of inflammatory diseases that manifest symptoms outside of the gut, such as allergy and asthma,[29] obesity and diabetes,[30] hypertension[31] and within the gut tissue (for example, inflammatory bowel disease and colorectal cancer).[32,33] During intestinal inflammation (that is, ulcerative colitis), the effector immune response toward the commensal bacteria is damaging to the protective mucosal layer in its efforts to neutralize opportunistically pathogenic bacteria.[34] Unfortunately, we do not understand the specificity of these neutralizing effects on specific strains and they probably affect both pathogenic and beneficial strains.

EXERCISE AND THE INTESTINAL MICROBIOME

It is now well established the exercise training initiates significant changes in the gut microbiome (genetic characterization of the microbiota) in animal models. A handful of studies indicate that exercise alters both the bacterial community structure and numerous taxa that are associated with host health.[35–37] Exercise can also induce gut microbial transformations in response to unfavorable stimuli or conditions, including high-fat diet (HFD),[38,39] toxic substances[36] and experimental diabetes.[40] Changes in the microbiome by exercise training have also been implicated in other metabolic and behavioral changes (that is, gut peptides and cognition).[38,41] The gut microbiome is also sensitive to the modality of exercise and the time in life when an individual begins exercise training.[35,37] Only one study to date has explored the effects of exercise training in humans.[42] Despite its cross-sectional design, this study provides intriguing evidence that exercise and/or fitness level is an important factor in the regulation of the gut microbiota in humans. Longitudinal studies are needed to definitively conclude whether exercise affects the human microbiome.

In animals, the first study to examine the effects of exercise on the microbiome demonstrated that 5 weeks of VWR in rats altered the composition of the ceca microbiome compared with sedentary controls.[43] The authors used temperature gradient gel electrophoresis banding to show that five out of seven of the exercised rats had ceca bacterial clusters distinct from that of the sedentary rats. Further evaluation of banding profiles revealed changes in two butyrate-producing bacteria (for example, SM7/11 and T2-87) and a twofold increase in the concentration of butyrate in the cecum induced by VWR. Butyrate, a short-chain fatty acid, is the preferred source of energy for colonic epithelial cells and has been shown to have numerous benefits on host intestinal function including the regulation of satiety, insulin sensitivity and inflammation.[44]

Choi et al.[36] tested the effects of 6 weeks of VWR on the microbiome of healthy mice and mice that were administered polychlorinated biphenyls (PCBs). PCBs are toxic, oncogenic environmental toxins that are found in many industrial products and are thought to have modulating effects on intestinal function.[39] They found that exercise significantly attenuated PCB-induced

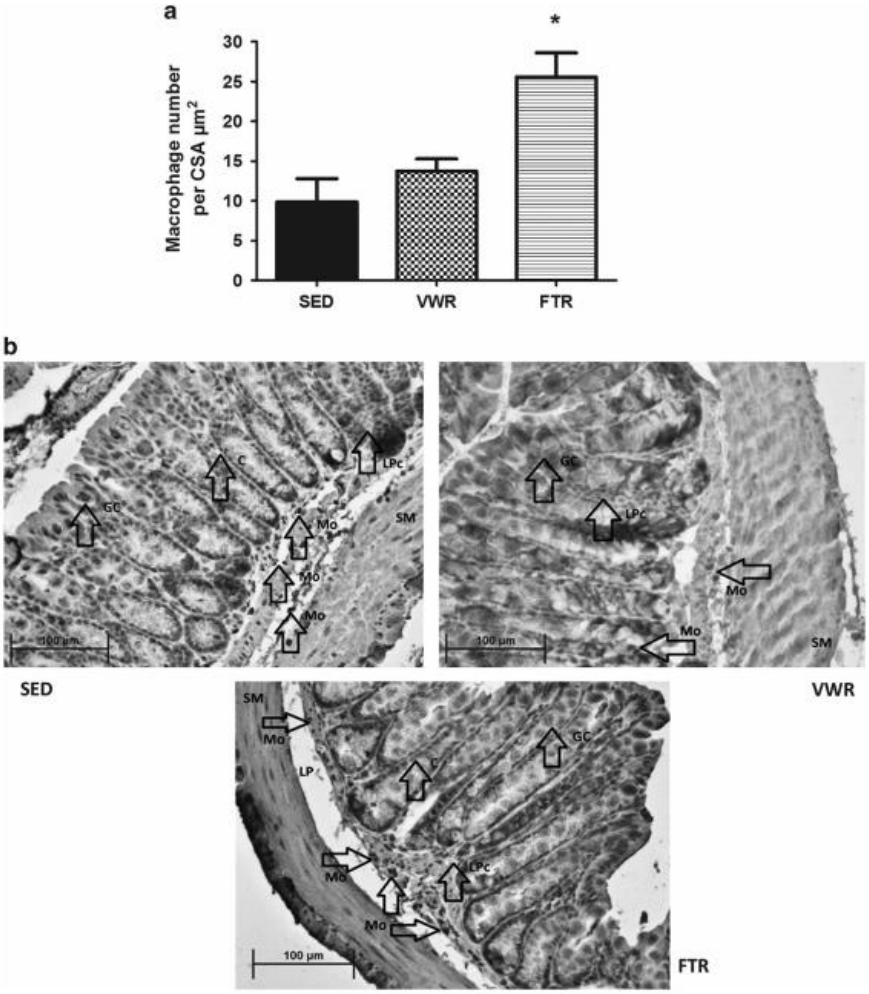

Figure 1 (a) Effects of VWR and FTR on macrophage presence in colon sections. FTR had a significantly greater resident macrophage population compared with SED and VWR. Data reported as mean ±s.e.m. of macrophage numbers per µm2 of colon tissue (SED: n =4; VWR: n =5; FTR: n =5); *Significance at P<0.05. (b) Effects of VWR and FTR on macrophage presence in colon sections. Slides SED, VWR and FTR are representative colon cross-sections (3 µm; ×40 magnification) probed for macrophage (F4/80+) cells and counterstained with Mayer's hematoxylin. C, colonocytes; GC, goblet cells; LP, lamina propria; LPc, lamina propria cells (consisting of fibroblasts, epithelial cells, lymphocytes and stromal cells); Mo, macrophages; SM, smooth muscle.

richer microbial diversity in the exercised mice that received PCBs compared with sedentary mice that received PCBs.[39] Interestingly, 67 taxa of bacteria were found to be unique to exercised mice, whereas 26 taxa were detected only in sedentary mice, regardless of PCB alterations.[36] Of these taxa altered by exercise alone, one-half were represented from order Lactobacillales. Lactobacilli have beneficial properties that support colon health and immune function,[45–47] and improve barrier function in intestinal disorders.[48,49] Multiple species of this genera have been used to effectively treat symptomology associated with irritable bowel syndrome,50 diarrhea51 and ulcerative colitis.[52]

We followed up the sequencing data presented by Choi et al.[36] and used targeted quantitative PCR with a universal lactobacilli primer. Similarly, we witnessed a significant, sevenfold increase in the relative presence of lactobacilli in distal colon mucosal scrapings of VWR mice when compared with SED and FTR mice: VWR versus SED (t_{13} = 5.9, *P = 0.004) and VWR versus FTR (t_{13} = 5.9, *P = 0.004), where *denotes significance of P<0.05 (SED, n = 10; FTR, n = 9; VWR, n = 5; Figure 2). We speculate that the greater presence of these bacteria on the mucosal layer after VWR could result in reduced inflammation during DSS-induced colitis through increased shortchain fatty acid production,[53] reduced nuclear factor-κB activation and pro-inflammatory cytokine production by intestinal epithelial cells[54] and macrophages,[48] and/or restored mucosal barrier function by reducing the colonization and potential translocation of opportunistic pathogens.[45]

Changes in the microbiota have also been observed in

mice fed a HFD. In collaboration with our laboratory, Kang et al.[38] demonstrated that forced wheel running altered numerous bacterial taxa, both alone and in combination with a HFD, some of which were strongly associated with improved cognition. However, exercise was not able to rescue the anxiety-like effects initiated by the HFD. Overall, these data point to exercise and diet orthogonally altering the microbiome, and point to multiple host–microbe interactions at play in diet and/or exercise interventions. Evans et al.[39] also observed multiple phyla and family level changes in the microbiome by VWR, both alone and in combination with a HFD. Notably, VWR reduced levels of Erysipelotrichaceae and Turicicibacteraceae, families of bacteria that are associated with obesity and gut inflammation, respectively, in humans.[55,56] Members of the Turicicibacteraceae family have been shown to have a close relationship with the host immune system. For instance, it was shown that populations of Turicibacter spp. were completely absent in two immunodeficient mouse models (innate immune deficient (for example, CD45 phosphatase deficient) and B- and T-cell deficient (for example, RAG))[27] compared with wild-type mice. Moreover, Turicibacter populations were abolished in Toll-like receptor-2 knockout mice when compared with wild-type mice.[27] Together these studies indicate immune- and disease-regulating capabilities of this bacterial taxa. With regards to exercise, recent data from our laboratory supports data observed by Evans et al.[39], in that voluntary exercise significantly reduced Turicibacter spp. populations in both the feces and ceca of mice, possibly indicating an immunoregulatory role of exercise through changes in microbial composition.[35] Despite these consistent findings between these two studies, the mechanisms by which exercise regulates certain microbial taxa, including Turicibacter, have not been elucidated.

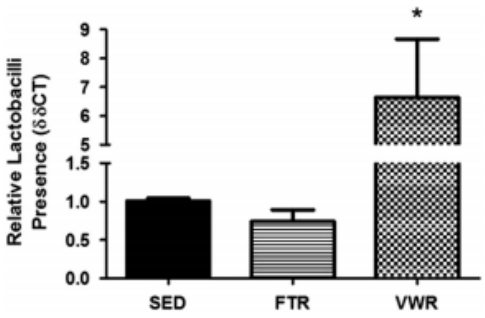

Figure 2 Effect of training on total lactobacilli presence in distal colon mucosal scrapings. Results are reported as relative fold expression from SED as the referent group. There is a significant (approximately sevenfold) increase in lactobacilli present in the mucosal scrapings of VWR mice compared with SED and FTR (SED, n = 10; FTR, n = 9; VWR, n = 5; * denotes significant difference over both SED and FTR $P<0.05$). Primers for Lactobacillus: (forward (LactoF) 5′-AGC AGT AGG GAA TCT TCC-3′, reverse (LactoR) 5′-CAC CGC TAC ACA TGG AG-3′) and general conserved bacterial DNA sequences for 16S ribosomal DNA as a normalizer (forward (8 F) 5′- AGA GTT TGA TCC TGG CTC AG-3′, reverse (1541 R) 5′- AAG GAG GTG ATC CAG CCG CA-3′) were used (Integrated DNA Technologies, IA).

The time of life when exercise training begins also appears to be an important factor in the regulation of the gut microbiome. Recently, Mika et al.[37] observed more significant microbial shifts in juvenile rats compared with their adult counterparts after VWR for 6 weeks. Moreover, the changes in microbial diversity and taxonomy were also associated with improved body composition in the juvenile, but not adult, rats. Another recent study indicated exercise capacity of an organism is an important regulator of the gut microbiome.[57] Interestingly, mice with a high capacity to run versus those exhibiting low-capacity running had different microbial characteristics following an HFD and VWR protocol. Moreover, these microbial differences between high capacity to run and low-capacity running were associated with disparate effects on fat mass and circulating nonesterified fatty acids following the combined intervention.[57] Taken together, these results indicate that the developmental stage on which an organism is exposed to environmental stimuli (that is, exercise) and the 'innate' capacity to exercise may be important for both the longterm characteristics of the microbiota and metabolic phenotype.[37]

The regulation of the microbiota may also depend on the modality and/or the conditions under which exercise takes place. To this point, our laboratory recently found that forced and voluntary exercise differentially alters the microbiome in both the cecum and feces of mice.[35] Similar to our findings of differential effects of FTR and VWR on DSS-induced colitis,38 we observed changes in taxonomy that point to divergent mechanisms regulating gut function. For example, we found that FTR significantly upregulated Ruminococcus gnavus,[35] a mucus-degrading bacteria that contains an intramolecular trans-sialidase enzyme and a Nan cluster, which together allow the bacteria to both degrade and consume terminal sialic acid residues on the mucin glycoprotein structure.[58] This 'selfish' trait employed by R. gnavus may allow it to penetrate the outer and inner mucosal layer, thus exposing the intestinal epithelial cells to immunogenic bacterial proteins and, in turn, exacerbate intestinal inflammation.[58] In fact, certain strains of R. gnavus and the total amount of intramolecular trans-sialidase are strongly associated with aberrant mucin dynamics and inflammatory bowel disease in humans.[58,59] In addition, the intramolecular trans-sialidase found in R. gnavus is also found in other well-known pathogenic bacteria such as Clostridium perfringens. It is important to note that the mucus-degrading characteristics of R. gnavus are in contrast to known beneficial mucus degraders such as Akkermansia muciniphilia, which need the synergistic action of several mucin-degrading enzymes and cannot consume terminal sialic acids on the mucin structure. It is possible that microbial changes induced by FTR including the upregulation of R. gnavus may result in aberrant mucus structure. Interestingly, chronic social stress alters the gut microbiome[60] and it was recently shown that water avoidance stress induced structural modifications of the mucin structure, favoring thicker bundling of mucin fibers and thus higher pore size for bacteria to penetrate.[61] Despite these findings, the regulation of mucin and bacterial dynamics are largely understudied in response to exercise, especially in

humans. Further research is needed in this area, especially considering the large number of individuals that suffer from IBS-like symptoms during and after strenuous exercise.[62]

Compared with FTR, VWR induced distinctive changes in both whole community structure and individual taxa within the mouse gut microbiome.[35] Of particular note, VWR increased the fecal and cecal content of the genus Anaerotruncus, a butyrate-producing taxa that colonizes the outer mucus layer of the colon and is phylogenetically related to Faecalibacterium prausnitzii, a known butyrate producer in the human colon.[35,55,63] Interestingly, butyrate producers such as Anaerotuncus and F. prausnitzii often feed off of lactate, acetate or other intermediate carbon structures from other strains of bacteria, indicating a cross-feeding of partial breakdown products. In relation to exercise, it is possible that exercise-induced alterations in lactate producers (for example, lactobacilli) and butyrate producers (for example, Anaerotruncus spp.) are related by a cross-feeding phenomenon. Despite our findings that exercise increased lactobacilli in the mucosal layer, it is important to note that we did not observe any changes in lactobacilli spp. in the feces or cecal contents after 16s rRNA sequencing.[35] These differences highlight the importance of investigating the microbiome in mulitiple areas (for example, cecum, feces and mucosal linings) throughout the GI tract, which can ultimately lead to more targeted approaches for researching microbial–host interactions. Unfortunately, the mechanisms by which exercise induces these changes in the microbiome are still largely unexplored.

Currently, only one cross-sectional study has examined the effects of exercise on the human gut microbiome. Clarke et al.[42] compared the microbiome from professional rugby athletes with that from age-matched sedentary counterparts.[42] Rugby players had a higher diversity of microbes and 22 distinct taxa represented in fecal samples compared with sedentary individuals. Of particular note, rugby players had a higher abundance A. muciniphilia, a beneficial mucin-degrading bacteria mentioned previously that is associated with leanness and improved insulin sensitivity in humans.[60,64] Despite these interesting correlations between physical activity and the microbiota, the study by Clarke et al.[42] is confounded by its cross-sectional nature and a lack of dietary and other external controls. Future human studies should include controlled, randomized, longitudinal designs that account for other external factors regulating the microbiota, such as diet and antibiotic usage among others.

CONCLUSION AND FUTURE DIRECTIONS

It is evident that strategies to promote healthy intestinal function are of great importance for overall physiological wellness, inflammatory disease prevention and treatment. Understanding the nature of immune–gut microbiome relationship require rigorous studies to uncover the mechanisms of interaction between GI immune cells, the gut microbiome and their relationships to disease. Human data from randomized controlled exercise trials are needed to fully understand resident immune cell shifts in the colon and changes in gut microbiome characteristics after exercise training and during psychological stress. Understanding the effects of habitual exercise on gut microbiota could lead to promising pathways of appreciating how exercise diminishes the risk of gut diseases (that is, inflammatory bowel disease and colon cancer), as well as systemic benefits of reducing systemic inflammation, improving insulin sensitivity, reducing blood pressure and aiding in the reduction of fat mass and increase of free fat mass. Future studies designed to understand exercise induced changes in the gut microbiome must consider past studies that have employed various sequencing techniques (for example, temperature gradient gel electrophoresis, pyrosequencing and Illumina platforms). These differences in analysis platforms may be responsible for variability and sometimes lack of cohesive results between studies. It is our opinion that future work examining the effects of exercise on the microbiota should incorporate targeted proteomic and metabolomic approaches along with whole community genomic sequencing. These will ultimately provide more comparable data sets and help to establish a more complete dialogue regarding the relationship between exercise, the gut microbiota and health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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