High-intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity

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Denou E, Marcinko K, Surette MG, Steinberg GR, Schertzer JD. High-intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity. Am J Physiol Endocrinol Metab 310: E982–E993, 2016. First published April 26, 2016; doi:10.1152/ajpendo.00537.2015.—Diet and exercise underpin the risk of obesity-related metabolic disease. Diet alters the gut microbiota, which contributes to aspects of metabolic disease during obesity. Repeated exercise provides metabolic benefits during obesity. We hypothesized that high-intensity interval training (HIIT) would counteract high-fat diet (HFD)-induced changes in the microbiota without altering obesity in mice. Compared with chow-fed mice, an obesity-causing HFD decreased the Bacteroidetes-to-Firmicutes ratio and decreased the alpha diversity in the cecal microbiota. HIIT increased the Bacteroidetes/Firmicutes ratio and decreased the alpha diversity and Bacteroidetes/Firmicutes ratio of the distal gut and fecal microbiota during diet-induced obesity. Exercise training increased the predicted genetic capacity related to the TCA cycle among other aspects of metabolism. Strikingly, the same microbial metabolism indexes that were increased by exercise were all decreased in HFD-fed vs. chow diet-fed mice. Therefore, exercise training directly opposed some of the obesity-related changes in gut microbiota, including lower metagenomic indexes of metabolism. Some host and microbial pathways appeared similarly affected by exercise. These exercise- and diet-induced microbiota interactions can be captured in feces.

Gut microbiome; exercise and oxidative metabolism; obesity; insulin resistance; diabetes

The mammalian gut contains a large and diverse community of bacteria that have been implicated in infectious and chronic diseases, which can manifest in the gastrointestinal tract and systemically (9, 16, 31). Colonization of the gut is influenced by complex environmental factors that include host genetics, age, diet, lifestyle, diseases, and antibiotic use (12, 15, 53). The reciprocal relationship between the gut microbiota and host metabolism can influence disease risk. Understanding these connections may yield avenues to manipulate resident mi-
lower in the small intestine (46). Exercise training is also linked to functional changes in the gastrointestinal tract, such as increased mouth to colon transit time without altering intestinal absorption (27). It is important to characterize exercise-induced alterations in the intestinal microbiome as a first step toward understanding whether microbial changes are responding to the stress of exercise and whether microbes are related to functional outcomes.

Endurance exercise training alters a myriad of host responses in both lean and obese individuals, including improvements in insulin sensitivity and increased cardiorespiratory fitness, effects associated with increased aerobic respiration (V\textsubscript{O\textsubscript{2}}) (52) and muscle mitochondrial enzymes such as those involved in the tricarboxylic acid (TCA) cycle (28, 29). High-intensity interval training (HIIT) has been shown to induce very similar metabolic effects to that elicited by endurance exercise training, including improvements in insulin sensitivity (25, 36). The mechanisms by which HIIT improves insulin sensitivity are not well understood, since obesity status is usually not altered, and recent studies in HFD-fed mice have demonstrated that HIIT improves both adipose and liver insulin sensitivity without changing body mass or adiposity (38). Remarkably, these changes in liver and adipose tissue insulin sensitivity occurred independently of liver lipid content or adipose tissue inflammation, suggesting that other factors may have been responsible for the insulin-sensitizing effects of HIIT.

Given the known connections between the gut microbiota and insulin sensitivity, we sought to determine whether HIIT alters the microbiota in mice after the establishment of obesity. We assessed whether HIIT influenced the taxonomy and predicted functional metagenomics of the murine gut and fecal microbiota during a HFD. The results show that HIIT opposed some of the effects of a HFD to reduce the predicted metabolic genetic capacity of the fecal microbiota independently of obesity. These data suggest that HIIT alters similar metabolic pathways in both the host and microbiota and raises the possibility that this type of exercise training may elicit some of its beneficial effects on metabolism through alterations in the gut microbiome.

MATERIALS AND METHODS

**Mice.** All experiments were approved by the McMaster University Animal Research Ethics Board (Hamilton, ON, Canada). All mice were born at McMaster University. Littermate mice were randomly assigned to exercise trained or untrained conditions using male offspring from a given in-house breeding pair of C57 BL/6 mice. This resulted in a mix of different mothers, breeding pairs, and cages in each experimental condition, which was done to limit any environmental or inherited influence on the results obtained for microbiota analysis. Eight-week-old male mice were maintained on a 12:12-h light-dark cycle and fed a HFD (45% kcal from fat, D12451; Research Diets, New Brunswick, NJ) for 12 wk. After 6 wk of HFD feeding, mice were either exercise trained (described in detail below) or left untrained. Untrained mice were exposed to the treadmill environment for equal periods of time as the exercise trained group. After a 3-day acclimation to treadmill running (19), exercise capacity was measured using a graded exercise test during which mice began running at 8 m/min on a 5% grade and treadmill speed was gradually increased by 1 m/min every 2 min until exhaustion, as described previously (38, 54). This test was also repeated after 6 wk of exercise training, which involved treadmill running 3 days per week for 1 h each day, as previously described (38). Specifically, the treadmill running involved running for 2 min at 17 m/min at a 5% grade (100% of average pretraining maximal running speed/exercise capacity) and then resting for 2 min. The treadmill running speed was increased by 1 m/min per week, so that by the end of the training protocol mice were running at 22 m/min. After 6 wk of exercise training, and 24 h after an exercise session, a 6-h fasted insulin tolerance test (ITT) was conducted. Tail vein blood glucose was measured using a glucometer during an insulin tolerance test (1 IU/kg), and epididymal adipose tissue was removed and massed, as described (30, 51). Two additional groups of mice were included as a comparison for microbiota analysis. Littermate male mice from multiple in-house breeding pairs were randomly assigned to standard chow diet feeding (n = 7 mice) or at 6–7 wk of age a subset of mice was fed the HFD (n = 9 mice) for 12 wk. The purpose of these additional groups was to provide context to the comparison between untrained and HIIT gut microbes.

**Microbiome sampling.** Feces were collected directly from the anus of mice into sterile tubes, which were immediately snap-frozen in liquid nitrogen before the mice were placed on the treadmill. Before repeated exercise training was initiated, the acute effects of a single exercise session were assessed by collecting feces within 1 h and 1 wk after the initial graded exercise capacity test. To test the chronic effects of repeated exercise training, fecal samples were collected 3 days after the last exercise training session following 6 wk of exercise training, with gut segments collected the following week 1 day after the final exercise session. At the completion of the 6-wk exercise training protocol, the duodenum plus jejunum, ileum, cecum, and colon were snap-frozen in liquid nitrogen and stored at −80°C. Fecal pellets were removed before processing any intestinal segment.

**Bacterial profiling.** Genomic DNA was extracted from fecal and gut segment samples. PCR amplification of the variable 3 (V3) region of the 16S rRNA gene was done on each sample, which included Illumina-compatible adapter sequences and barcoding for multiplexing. DNA products of this PCR amplification were sequenced using the MiSeq platform followed by preliminary analysis by the McMaster Genome Center (McMaster University). A custom in-house pipeline was used to process the FASTQ files as described (17). Cutadapt was used to trim sequences beyond the 16S rRNA V3 region, and PANDAseq was used to align paired-end sequences (39, 40). AbundantOTU+ grouped reads into operational taxonomic units (OTUs) based on 97% similarity (41, 60). Taxonomy was assigned to OTUs Ribosomal Database Project (RDP) classifier in Quantitative Insights Into Microbial Ecology (QIIME) (6) against the 2011 version of the Greengenes reference database (18). QIIME was used to calculate the diversity within communities (alpha diversity) and between communities (beta diversity), as previously described (6, 17). At the genus level, OTUs were assigned to the corresponding genus and represented to the closest root of the phylogenetic tree. This can result in different OTUs being assigned to the same classification. Principal coordinates analysis (PCoA) used the Bray–Curtis dissimilarity values to position the points relative to each other. Sequencing characteristics are described in Table 1. Prediction of metagenome functional content from the 16S rDNA library was developed using PICRUSt software, and PICRUSt predictions were categorized as levels 1 to 3 into KEGG pathways (34). QIIME was used to visualize the predicted functions within KEGG pathways.

**Statistical analysis.** Results were analyzed by an unpaired, two-tailed Student’s t-test where two means are compared or ANOVA (for more than two means) using GraphPad Prism 6 software. Subsequently, false discovery rate (FDR) was accounted for via implementation of the Benjamini-Hochberg multiple testing adjustment procedure using R, where FDR-corrected P values were estimated for all taxonomic data or predicted metagenomic data within a specific PICRUSt level. Statistical significance was accepted at P < 0.05 after adjustment for FDR.
Table 1. QIIME 16S rDNA sequencing analysis

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Untrained Mouse Gut Segments</th>
<th>Trained vs. Untrained Mouse Feces</th>
<th>Chow- vs. HFD-Fed Mouse Feces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of samples</td>
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<td>14</td>
<td>33</td>
</tr>
<tr>
<td>Minimum sequences count</td>
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<td>92,644</td>
<td>32,649</td>
</tr>
<tr>
<td>Maximum sequences count</td>
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<td>131,501</td>
<td>76,187</td>
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<tr>
<td>Median sequences count</td>
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<td>100,643</td>
<td>42,021</td>
</tr>
<tr>
<td>Normalized sequences*</td>
<td>9,880</td>
<td>92,640</td>
<td>32,640</td>
</tr>
<tr>
<td>Number of 97% phylotypes (genus level-assignable)</td>
<td>335</td>
<td>187</td>
<td>167</td>
</tr>
</tbody>
</table>

*Numbers of sequences in each dataset (of a given sample) were normalized by rarefaction to allow for intra-sample comparisons of the datasets.

RESULTS

HFD alters the gut and fecal microbiota. Consistent with previous findings (17, 58), we observed a lower Bacteroidetes/Firmicutes ratio in the distal gut and feces in HFD-fed mice compared with chow-fed mice (Fig. 1A). This comparison of chow-fed mice (average body mass 32 ± 0.5 g) to HFD-fed mice (average body mass 50 ± 0.7 g) was separate from all exercise-related experiments. Compared with chow-fed mice, HFD-fed mice were insulin and glucose intolerant (data not shown). We found no evidence that the type of diet changed the overall alpha diversity (data not shown), but we found lower alpha diversity (i.e., Shannon index) within the phylum Bacteroidetes in both the colon and feces of HFD-fed mice (Fig. 1B). Additional differences in the colonic and fecal microbiome taxonomy from chow-fed vs. HFD-fed mice are shown in Supplemental Table S1 (supplemental materials are found with the online version of this paper).

Exercise training during obesity improves insulin tolerance independently of adiposity. Mice were on an obesity-causing HFD for 12 wk with HIIT during the final 6 wk (Fig. 2A). HIIT was initiated after the establishment of obesity, and this type of repeated exercise training did not alter body mass in HFD-fed mice (Fig. 2A). As expected, HIIT increased the time to exhaustion and running speed at the completion of a graded exercise test (Fig. 2, B and C). Exercise training also increased insulin tolerance, but it did not alter fasting blood glucose and did not alter epididymal adipose tissue mass in HFD-fed mice (Fig. 2, D–F). We (38) recently demonstrated that this HIIT exercise protocol increased oxygen consumption (V\textsubscript{O\textsubscript{2}}), carbon dioxide consumption (V\textsubscript{CO\textsubscript{2}}), respiratory exchange ratio (RER), food intake, and water intake in HFD-fed mice. We also published that this HIIT exercise protocol does not alter body mass, whole body adiposity, adipose mass, liver mass, or heart mass (38). Importantly, we made every effort to control for different environmental microbial exposures in the mice, including placing all of the untrained mice on the exercise treadmill (which was turned off) for an equal duration that corresponded to each exercise training session.

Exercise training during obesity alters the microbiota in a gut segment-dependent manner. Exercise training of HFD-fed, obese mice increased the overall alpha diversity of the microbiota in the colon (Fig. 3A). Exercise training increased the alpha diversity within the phylum Bacteroidetes in both the cecum and colon (Fig. 3B). Exercise training increased the Bacteroidetes/Firmicutes ratio in the cecum, and a similar trend (P = 0.06) was seen in the colon (Fig. 3C). No clear pattern of exercise training-related changes could be seen after PCoA (Fig. 3D). Exercise training did not significantly alter any other phylum-level microbiota characteristics as depicted by relative quantity of each phylum in various gut segments (Fig. 3E). These data indicate that exercise training opposed the effects of the HFD, since a HFD decreased, but exercise training increased, the Bacteroidetes/Firmicutes ratio and diversity within Bacteroidetes in the distal gut.

At the OTU level, exercise training during the final 6 wk of the HFD had more profound effects in the distal gut. Figure 4A depicts the average relative abundance of genera in various gut segments of HFD-fed mice that that were exercise trained or untrained. Figure 4B–E, depicts all of the statistically signif-
significant genus-level changes associated with exercise that occurred in at least one of the gut segments after correction for FDR. Based on the relative quantity of each OTU that was detected, exercise training was associated with a significant difference in 1 OTU in the duodenum plus jejunum [Actinobacteria (c)] and 1 OTU in the ileum (Lactobacillus) compared with 3–5 significantly different OTUs in the cecum or colon (Fig. 4, B–E). Also at the OTU level, exercise training increased Bacteroidales (o) in both the cecum and colon (Fig. 4, D and E). Overall, this data shows that repeated exercise training altered the constituents of the microbiome more profoundly in the distal gut during HFD-induced obesity and that HIIT consistently increased Bacteroidales (o) in both the cecum and colon of obese mice.

Exercise training during obesity alters the fecal microbiota. Feces are easier to obtain for biomarker assessments, and use of feces allows comparison within the same individual/mouse across time. Hence, we analyzed the feces of all HFD-fed mice...
before exercise (i.e., PreTreadmill) compared with samples collected after 6 additional weeks from HFD-fed mice that were exercise trained and age-matched HFD-fed untrained mice. Analysis of the fecal samples collected before mice were allocated to trained or untrained groups (i.e., direct comparison within the PreTreadmill groups) showed that the exercise training-induced differences in microbiota characteristics were not derived from an existing difference in these microbiome measurements before mice initiated exercise training (data not shown). Within-subjects analysis at the phylum and genus levels (Fig. 5, A and B) revealed that 6 wk of exercise training significantly increased only 1 OTU in the feces. Bacteroidales (o) was higher in exercise-trained vs. pre-treadmill fecal samples (Fig. 5C). This is consistent with exercise training-induced effects in the cecum and colon. Exercise training increased the alpha diversity, as measured by a higher Shannon index in trained vs. untrained fecal samples (Fig. 5D), but it did not change the Shannon index within Bacteroidetes (data not shown). Exercise training also increased the Bacteroidetes/Firmicutes ratio compared with pre-treadmill fecal samples (Fig. 5E). Again, there was no discernable pattern based on PCoA of exercise-trained, untrained, and PreTreadmill fecal samples (data not shown). These data indicate that exercise training increased Bacteroidales (o) and diversity in the feces, which is reflective of exercise-induced changes in distal gut of HFD-fed mice.

We next sought to determine whether these changes in the taxonomy of the microbiota during repeated exercise training were independent of an acute bout of exercise. In feces collected within 1 h and 1 wk after a single acute bout of exercise, we found no phylum-level changes (Fig. 6, A and B). Only one genus (Lactococcus) was decreased 1 h after acute exercise (Fig. 6C), and this change did not persist 1 wk after acute exercise. Finally, there was no change in the overall alpha diversity (Shannon index) or alpha diversity within Bacteroidetes or Bacteroidetes/Firmicutes ratio of the feces at 1 h or 1 wk after a single exercise session compared with pre-treadmill values (Fig. 6, D–F). Overall, these results show that the changes observed in repeated exercise training could not be explained by the effect of a single exercise session.

Exercise training during obesity alters the predicted function of the fecal microbiota. We next sought to determine whether exercise training was associated with predicted changes in the genetic capacity for microbial functions in the feces. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) analysis showed a significant increase in the microbial genetic material that is KEGG-annotated to metabolism after exercise training (Fig. 7A). This directly opposes a decrease in the genetic capacity related to metabolism during a HFD compared with chow diet (Fig. 7B). HFD also increased the predicted genetic capacity related to Environmental IP (Fig. 7B). Further analysis revealed that exercise training altered predicted pathways only within the KEGG pathways assigned to metabolism. Exercise training increased the fecal microbial genes predicted to be involved in glycan biosynthesis and metabolism, carbon fixation, and the TCA/citrate cycle (Fig. 7C). Again, this directly opposes a subset of the changes during a HFD where all of these KEGG-assigned metabolic pathways were decreased compared with chow fed mice (Fig. 7D). A single acute exercise session did not alter these or any predicted metagenomic characteristics (data not shown).

The effects of HFD (vs. chow diet) included over 30 changes within the metabolism annotation (Supplemental Table S1). Therefore, the effects of diet on the microbiota were more extensive compared with exercise. Nevertheless, our results show that exercise and an obesity-causing diet shift the predicted metagenomic characteristics of the fecal microbiota in opposite directions. Furthermore, our results show that exercise during diet-induced obesity directly opposes some of the obesity-related functional characteristics of the microbiota despite no change in body mass.

**DISCUSSION**

Diet and exercise are two factors involved in obesity. Obesity is associated with altered gut microbiota. We show that HIIT can oppose some of the microbiota changes characteristic of diet-induced obesity even though there is no change in body mass or adipose tissue mass during this type of exercise training. These exercise-related changes in the microbiota include expansion of the predicted genetic capacity related to various pathways in metabolism.

Diet-induced changes in the microbiota have emerged as a contributor to host metabolism relevant to obesity-related disease. Gut-derived microbial factors can influence metabolic disease characteristics (4, 10). Multiple diet-related factors influence obesity, including caloric intake and macronutrient composition. Diets high in fat promote obesity and alter the taxonomic and metagenomic characteristics of the gut microbiome (58). The characteristic changes in the gut microbiota during an obesity-causing HFD include a lower relative level of Bacteroidetes and a relative expansion of Firmicutes (35, 59). A high-fat/sugar diet is the dominant factor influencing the gut microbiota compared with host genetics (7). Furthermore, the fecal microbiota from lean and obese twins can influence fat mass when transferred to germ-free mice (50). Therefore, diet and/or obesity appear to be a powerful factor influencing the metabolic effects of the microbiota. Our results confirm that diet (i.e., HFD vs. chow diet) is a dominant factor in shaping the microbiota, including its predicted functional genetic characteristics. Diet interacts with many host and environmental factors that can potentially influence the microbiota and obesity. We sought to determine whether exercise was a factor that could influence or overcome dietary shifts in the microbiota. Exercise can counterbalance many host metabolic processes during obesity, but little is known about how exercise influences the microbiota during diet-induced obesity.

We found that exercise training (i.e., HIIT) during obesity promoted changes in the distal gut and fecal microbiota that

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**Fig. 4. Genus level changes of gut microbiota associated with repeated exercise. Average relative abundance of genera in various gut segments of HFD-fed untrained and exercise-trained mice (A). OTUs significantly different in microbiota from any gut segment are shown from exercise-trained vs. untrained HFD-fed mice (B–E). Significant differences are noted in boldface for taxonomic classification in duodenum plus jejunum (A), ileum (B), cecum (C), and colon (D). A: data are mean values; B–E: data are box-and-whisker plots. *P < 0.05 trained vs. untrained. Nos. of mice analyzed for each condition are shown in brackets.**
were opposite to those characteristic of obesity and/or a HFD. For example, exercise training increased the Bacteroidetes/Firmicutes ratio and also increased the alpha diversity (within the Bacteroidetes phylum) of the microbiota. HIIT could overcome the influence of a HFD in the distal gut and feces, since exercise training opposed some of the taxonomic and predicted metagenomic changes caused by diet-induced obesity. Altered host obesity/adiposity appears not to be a major driver of these exercise-induced changes in the microbiota, since the type of exercise used in our study did not alter body mass or adipose tissue mass during the HFD. Adaptations to repeated exercise sessions appear to be required for diversity and taxonomic and predicted metagenomic microbiota characteristics, since acute exercise did not alter these indexes. A limitation of our study

**Fig. 5.** Taxonomic changes in the fecal microbiome associated with repeated exercise. Average relative abundance of the major phyla (A) and genera (B) in feces of HFD-fed untrained and exercise-trained mice. Depiction of the only significantly different genus-level change [Bacteroidales (o)] in the feces (C). Alpha diversity (Shannon index) in exercise-trained vs. untrained vs. PreTreadmill conditions in feces from HFD-fed mice (D). Relative Bacteroidetes/Firmicutes ratio in exercise-trained vs. untrained vs. PreTreadmill conditions in feces from HFD-fed mice (E). A and B: data are mean values; C: data are box-and-whisker plots; D and E: data are means ± SE. #P < 0.05 Trained vs. Untrained. *P < 0.05 Trained vs. PreTreadmill. Ttaxonomic data for the PreTreadmill condition are presented in Fig. 6. Nos. of mice analyzed for each condition are shown in brackets.
was the inability to segregate improved insulin tolerance caused by repeated exercise as a factor that could contribute to alterations in the microbiota.

There is evidence mounting that sustainable perturbations to the microbiota can alter obesity-related metabolic disease characteristics. For example, a low dose of antibiotics given during an early-life window can promote increased adiposity (11). The transient early-life dysbiosis caused by certain antibiotics appears to interact with dietary stress, since it magnifies the obesity-causing effect of a HFD (15). Antibiotics can also promote intestinal dysbiosis that is sufficient to accelerate diabetes (3). Immune signals are positioned to connect microbial dysbiosis to disease characteristics (2). Diet is a powerful factor influencing the microbiota, which can influence immune underpinnings of disease (37). Unraveling the compartmentalized responses and connections among diet, dysbiosis, metabolism, and immunity is a complex challenge (43). We (8) have recently shown that diet-induced changes in intestinal immu-
nity do not necessarily parallel immune responses in adipose tissue. It is beyond the scope of this work to determine the underlying immune or endocrine signals that are a potential cause or consequence of exercise and diet-induced changes in the microbiota. Future goals of this type of work include assessments of immune cell populations and inflammatory or endocrine mediators in the gut and metabolic tissues. This will be important, since there is a reciprocal relationship between inflammatory mediators and cellular energy sensors involved in exercise responses (22, 55). These connections could also engage adipokines and myokines and changes in bile acid metabolism and could involve increased food/water intake coincident with exercise training. Intriguingly, different dietary lipids can promote dysbiosis during aging and infectious colitis.

Fig. 7. Predicted functional metagenomic changes in the fecal microbiome associated with diet and exercise. Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) was used to calculate and the relative proportion of genes predicted to be present in various KEGG pathways in feces from exercise-trained and untrained mice all fed a HFD (A). This was compared with the relative proportion of genes predicted to be present in various KEGG pathways in feces from chow-fed and HFD-fed mice e not exercise trained (B). Depicted are significant differences in feces from exercise-trained and untrained mice (C) and side-by-side comparison of a subset of those significant differences within the metabolic pathways in feces from chow-fed and HFD-fed mice (D). Data are means ± SE. *P < 0.05 Trained vs. Untrained; #P < 0.05 Chow vs. HFD. Nos. of mice analyzed for each condition are shown in brackets.
The evidence is mounting that exercise is a perturbation that can influence the microbiota. Our most consistent finding regarding taxonomy was an exercise-training-related increase in the levels of *Bacteroidales* (α) in distal gut (cecum, colon) and feces. In HFD-fed mice, we also found an increase in *Dorea* in the cecum and colon, which is consistent with other groups that employed forced treadmill running and found increased *Dorea* in the cecum and feces in chow-fed mice (1). Another previous study found by investigating the interaction of exercise and nonexercised mice that were chow fed vs. HFD-fed that exercise and diet each altered the microbiota independently (32). This type of exercise caused decreased body mass in HFD-mice, which is different from the HIIT protocol in the current study. In fact, the mode of exercise could be very important in dictating changes in microbiome characteristics. As expertly reviewed, rodent models using different types of exercise have shown different gut microbiome characteristics (13). This may be related to differential immune responses in the gut. For example, macrophage number is higher in the colon of mice after forced treadmill exercise, an effect that does not occur with voluntary wheel running (13). This is an intriguing gut immune response that may relate to the exercise-related expansion of lactobacilli in the distal colon during voluntary running, but not forced treadmill running (13). These gut immune and microbe changes correspond to functional outcomes such as increased inflammatory score and mortality due to forced treadmill running if there is an additional stress such as a model of colitis in mice (14). It remains to be determined what aspects of metabolic disease, such as obesity, hyperglycemia, or even specific dietary components interact with different modes of exercise to influence the microbiome. This seems worthwhile, since it has already been shown that 6 wk of exercise increased *Bifidobacterium* in the cecum of control mice but not in hyperglycemic leptin receptor-deficient (*db/db*) littermate mice (33).

The effects of exercise training on the microbiota during a HFD diet in our study were relatively small compared with changes in diet or other stimuli that can cause dysbiosis such as antibiotics. This was not that surprising, and we chose to characterize exercise because of the overwhelming evidence of the host metabolic changes (and health benefits) induced by exercise. This descriptive information may set the stage for understanding how to assess whether microbes play a role in the metabolic health benefits of exercise during obesity. Furthermore, the altered microbial signatures may be able to be used as a biomarker of exercise status or responsiveness. Our data support investigation of exercise-induced metagenomic characteristics in the feces.

Exercise is well known to increase oxidative capacity, mitochondria, and proteins involved in the TCA cycle (28, 29, 47). Our results show that exercise training increased the predicted metagenomic capacity for metabolism and the TCA cycle in the fecal microbiota. Our data only provide a prediction of the genetic capacity of the microbial community for these KEGG pathways. Caution is warranted in assuming that these genetic indexes influence the same host pathways by producing chemical messengers or altering the metabolism of substrates. It is not yet clear which of the many host effects of exercise relate to microbiota changes. Nevertheless, it is enticing to speculate about the exercise-induced functional changes in the microbiota, which occurred only in the metabolism annotation. The observed changes in poorly classified glycan biosynthesis could be linked to the regulation of mucin O-glycan or biosynthesis of lipopolysaccharide or peptidoglycan. All of these pathways have been implicated in aspects of host metabolic disease (4, 10, 17, 21, 51, 56). The observed changes in carbon fixation and the TCA cycle pathways could be related to acetyl-CoA and short-chain fatty acid regulation, which have been implicated as microbial ligands/metabolites that influence host metabolism (5). For example, exercise has been shown to increase cecal butyrate levels in rats (42).

HFD had a more profound effect on the predicted metagenomic characteristics of the fecal microbiota, which corresponds to diet dominating other factors (7). Intriguingly, all of the exercise-induced changes in predicted microbial function occurred in the opposite direction compared with those associated with a HFD. Our data suggest that exercise counterbalances a subset of the changes in microbial function caused by obesity or obesity-causing diets. It is not clear how repeated exercise training elicits a change in the microbiota of the distal gut, but intestinal motility should be considered. Moreover, exercise reduces blood flow to the colon to a much greater degree than other parts of the intestine. This reduced blood flow might equate to a hypoxic state in the resident microbiota, which could dictate expansion of microbes with a greater metabolic or suitable respiratory capacity. It is probable that the exercise-induced changes include bacteria where the respiratory chain proteins do not necessarily require oxygen as the terminal electron acceptor. Nevertheless, future studies testing different exercise regimens (low- and high-intensity training), altered intestinal blood flow, and hypoxia will be important to delineate whether these environmental cues alter the gut microbiome.

In summary, our results show that repeated exercise training can overcome a distinct subset of the changes in the distal gut and fecal microbiota caused by HFD-induced obesity, independently of changes in body mass or fat mass. In the fecal microbiota, an obesity-causing diet decreased, whereas repeated exercise training increased, several predicted metagenomic traits involved in metabolism, including the TCA/citric acid cycle. Exercise training is well known to regulate these host metabolic pathways, and it is enticing to speculate that the physiological response to exercise also includes changes in analogous microbial pathways.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
EXERCISE OPPOSES HIGH-FAT DIET EFFECTS ON THE MICROBIOME

AUTHOR CONTRIBUTIONS

E.D., K.M., and J.D.S. performed experiments; E.D., K.M., M.G.S., G.R.S., and J.D.S. analyzed data; E.D., K.M., M.G.S., G.R.S., and J.D.S. prepared figures; E.D., G.R.S., and J.D.S. edited and revised manuscript; E.D., K.M., M.G.S., G.R.S., and J.D.S. conceived and designed of research; J.D.S. drafted manuscript.

REFERENCES


