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Abstract

PURPOSE: Exercise is associated with altered gut microbial composition, but studies have not investigated whether the gut microbiota and associated metabolites are modulated by exercise training in humans. We explored the impact of six weeks of endurance exercise on the composition, functional capacity, and metabolic output of the gut microbiota in lean and obese adults with multiple-day dietary controls prior to outcome variable collection. **METHODS:** Thirty-two lean (n=18 [9 female]) and obese (n=14 [11 female]), previously sedentary subjects participated in six weeks of supervised, endurance-based exercise training (3 days/wk) that progressed from 30 to 60 minutes/day and from moderate (60% of heart rate reserve [HRR]) to vigorous intensity (75% HRR). Subsequently, participants subsequently returned to a sedentary lifestyle activity for a six week washout period. Fecal samples were collected before and after six weeks of exercise, as well as after the sedentary washout period, with 3-day dietary controls in place prior to each collection. **RESULTS:** β-diversity analysis revealed that exercise-induced alterations of the gut microbiota were dependent on obesity status. Exercise increased fecal concentrations of short chain fatty acids (SCFAs) in lean, but not obese, participants. Exercise-induced shifts in metabolic output of the microbiota paralleled changes in bacterial genes and taxa capable of SCFA production. Lastly, exercise-induced changes in the microbiota were largely reversed once exercise training ceased. **CONCLUSION:** These findings suggest that exercise training induces compositional and functional changes in the human gut microbiota that are dependent on obesity status, independent of diet and contingent on the sustainment of exercise. **Keywords:** exercise, gut, microbiota, microbiome, obesity, butyrate, human

INTRODUCTION

Environmental stimuli and behavioral practices can modulate the composition and functionality of the gut microbiota. For instance, diet (1), obesity status (2), and mode of birth delivery (3) have been shown to significantly contribute to the metabolic and immune-regulating capacity of the gut microbiome throughout the lifespan. Physical activity status in humans correlates with microbiota composition; however, the effects of endurance-based exercise training on the human gut microbiota have not been explored.

Despite the lack of research in humans, data from our laboratory and others indicate that exercise training can significantly modulate the gut microbiota in animal models (4-6). Moreover, exercise-induced changes in the gut microbiota have been correlated with changes in host physiology, including alterations in metabolism (7), immunity (5), and behavior (6). Exercise training also increases microbiota-derived short chain fatty acids (SCFAs) within the mouse gut (8). SCFAs are two to six carbon long fatty acids which serve as an energy source for a variety of tissues and have been shown to reduce inflammation (9), improve insulin sensitivity (10), and alter central nervous system morphology (11)

Among humans, Estaki et al. showed that cardiorespiratory fitness (VO_{2max}) was positively correlated with increased bacterial diversity and butyrate-producing bacteria (12), while Barton et al. described that the gut microbiome in athletes support higher turnover of macromolecules (carbohydrates and protein) and higher gut SCFA concentrations compared to sedentary controls (13). Despite offering intriguing associations between exercise and the gut microbiota, these studies are limited by their cross-sectional design because other factors that transiently alter the gut microbiota are not controlled. Thus, the present study was designed to assess gut microbial changes in a longitudinal design that controls for diet and antibiotic use.

The effects of exercise on the human gut microbiota have also not been investigated in the context of obesity. An obesity-associated gut microbiota can significantly contribute to host weight gain through enhanced energy harvest (14) and drive inflammation through reduced gut barrier integrity (15). Exercise training can contribute to improvements in obesity status and associated co-morbidities by increasing insulin sensitivity, reducing systemic inflammation (16), and improving VO_{2max} (17). Exercise training in mice has also been shown to limit gut barrier integrity disruptions and tissue inflammatory responses induced by obesity, offering evidence of a possible gut microbiota-mediated mechanism by which exercise improves outcomes associated with obesity (18). However, considering the wide-ranging effectiveness of exercise interventions in combating weight gain and obesity-associated co-morbidities in humans (19), a more comprehensive investigation into the biological systems that are responsive to both obesity and exercise training, including the gut microbiota, is needed.

In this study, we investigated whether six weeks of aerobic exercise training alters gut microbial communities and fecal SCFAs in previously sedentary lean and obese adults with controls in place to ensure consistent dietary patterns. We hypothesized that exercise training would stimulate orthogonal shifts in gut microbial communities dependent on BMI status and, lead to increased fecal SCFA levels as well as functional metabolic capacity for the gut microbiota to produce SCFA. Lastly, we also predicted that composition, functional capacity and metabolic output of the gut microbiota would return to baseline levels after an extended (6 week) reversion to sedentary activity.

METHODS

Participants and ethical approval

Thirty-two adult, previously sedentary females (n=20) and males (n=12) were recruited based on a lean or obese body mass index (BMI); lean (BMI < 25 kg/m^2; n=18; 9 female, 9 male) and obese (BMI > 30 kg/m^2; n=14; 11 female, 3 male). Baseline participant characteristics are shown in **Table 1**. This study was approved by the University of Illinois Institution Review Board, written informed consent was obtain for all participants and all procedures and protocols conformed to the standards of use of human participants in research as outlined in the Sixth Declaration of Helsinki..

Inclusion and Exclusion Criteria

Participants were 20-45 years of age, had a BMI < 25 kg/m^2 (lean) or a BMI > 30 kg/m^2 (obese) and were sedentary ($\leq$ 30 minutes of moderate or high intensity exercise per week and $\leq$ 10 aggregate Godin-Shepard Leisure Time Physical Activity Questionnaire (GSLTQ; Godin-Leisure) score. Subject medical history and medication use was assessed through questionnaire. Subjects that qualified for the study were free of metabolic and gastrointestinal disease, not pregnant or lactating, not taking medications that would impact bowel function, and not taking antibiotics for at least three months prior to or during the study.

Study Design

The study was conducted as a longitudinal design lasting 14 weeks. After two weeks of baseline testing, all subjects completed a six-week endurance-based exercise intervention. The exercise training period was followed by a six-week 'washout' period, during which participants were instructed to refrain from exercising. Fecal and blood samples were collected throughout the study, with 3-day dietary controls in place prior to each collection (see Figure, Supplemental Digital Content 1**,** Experimental Design, http://links.lww.com/MSS/B104).

Screening and Diet Control

At baseline, subjects came to the laboratory for four visits. During these visits, GSTLQs were administered to ensure participants were sedentary over a period of at least three months. Participants were asked to complete a 7-day dietary record, which included detailed descriptions of the types and amounts of foods and beverages consumed. Then, in consultation with a registered dietitian (RD), participants designed a 3-day food menu that consisted entirely of foods and drinks from the 7-day dietary recall. Participants were asked to follow this 3-day food menu prior to each fecal collection. Beyond the acute dietary control, subjects were also instructed to maintain overall dietary patterns, which included maintenance of alcoholic/caffeinated beverage consumption and continuation of any dietary supplement usage that occurred prior to the beginning of the study. Three-day menus were analyzed for macro- and micro-nutrient composition, food groups, and individual food item consumption (see Table, Supplemental Digital Content 2**,** Baseline dietary consumption, http://links.lww.com/MSS/B105) using Nutrition Data System for Research (NDSR 2014 version, University of Minnesota, Minneapolis, MN).

Exercise training protocol

The exercise intervention consisted of three supervised 30 to 60 minute, moderate-to-vigorous intensity (60-75% of heart rate reserve [HRR]) aerobic exercise sessions per week. Subjects chose from a cycle ergometer or treadmill during each exercise session. Percent of HRR was calculated as:

$$\text{percent (\%) of target intensity} \times (HR_{max} - HR_{rest}) + HR_{rest}$$

HR_{max} was determined during the baseline VO_{2max} test. During Week 1 of training, sessions were 30 minutes at 60% HRR. Training sessions for Week 2 lasted 45 minutes at a similar intensity.

Training sessions at Week 3 were increased to 60 minutes at 60% HRR. During Weeks 4-6 of training, there was an increase in intensity of 5% HRR per week, progressing up to 75% HRR for 60 minutes during Week 6. All participants were 100% compliant in completing necessary requirements for the exercise portion of the study.

Washout Period

Following post-training assessments, all participants were instructed to refrain from exercise for the next six weeks. To assess washout compliance, GSLTQs were again administered. Participants that scored below 10 were deemed to have returned to sedentary activity and were included in the washout analysis. A total of seven subjects (n=4 lean and n=3 obese) remained physically active during the washout (GSLTQ >10) and were not included in the washout analysis.

Fecal Collections

Fecal samples were collected at baseline (E0), following six weeks of exercise (E6), and following six weeks of return to sedentary activity (W6). All collections were preceded by the 3-day food menu. Subjects were instructed to bring samples into the laboratory within 30 minutes of defecation to ensure minimal degradation of volatile SCFAs. Researchers asked participants the time defecation to ensure sample was received within 30 minutes. Once received, a small portion of the sample (~0.5 grams) was aliquoted for SCFA analysis and the rest was stored at -80°C until future analysis.

Body Composition Assessments

Body composition was assessed using Dual Energy X-ray Absorptiometry (Hologic QDR 4500A, Bedford, Massachusetts) at E0, E6, and W6.

Maximal Oxygen Consumption (VO_{2max})

At baseline (E0) and after the exercise intervention (E6), participants completed a maximal oxygen uptake test to assess cardiorespiratory fitness (VO_{2max}) (Parvo Medics True Max 2400, Sandy, UT). This test involved walking or running to maximal exertion on a treadmill using the Bruce testing protocol as described previously (20). Heart rate (HR) was continuously recorded via wireless Polar Monitor (Polar Electro, Lake Success, NY) and the test ended upon volitional fatigue. VO_{2max} was verified if two or more of the following criteria were met: (1) Rate of Perceived Exertion > 18, (2) HR within 10 beats per minute (bpm) of age-predicted max HR, (3) a plateau in HR (< 3 bpm change) over the last two intensity stages, and/or (4) Respiratory Exchange Ratio (RER) of > 1.10.

Bacterial DNA isolation and 16S rRNA sequencing

Fecal bacterial DNA was extracted using the PowerLyzer PowerSoil DNA Isolation Kit (MOBIO Laboratories, Inc.) and quality was assessed via gel electrophoresis. The DNA library was constructed using a Fluidigm Access Array system in the Functional Genomics Unit of the Roy J. Carver Biotechnology Center at the University of Illinois at Urbana (UIUC). After library construction, a 300 bp region of the 16S rRNA gene (V4) were amplified using a two-step PCR amplification method modified from Muturi et al. (21). Briefly, DNA samples were diluted to 2 ng/µl and amplified with Roche High Fidelity Fast Start Kit and 20x Access Array loading reagent prior to PCR. Samples were amplified using the following Access Array cycling program 50 °C for 2 minutes (1 cycle), 70 °C for 20 minutes (1 cycle), 95 °C for 10 minutes (1 cycle), followed by 10 cycles at 95 °C for 15 seconds, 60 °C for 30 seconds, and 72 °C for 1minute, 2 cycles at 95 °C for 15 seconds, 80 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 1 minute, 8 cycles at 95 °C for 15 seconds, 60 °C for 30 seconds, and 72° for 1minute, 2 cycles at 95 °C for 15 seconds, 80 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 1minute, 8

cycles at 95 °C for 15 seconds, 60 °C for 30 seconds, and 72 °C for 1minute, and 5 cycles at 95 °C for 15 seconds, 80 °C for 30 seconds, 60 °C for 30 seconds, and The PCR was quantified on a Qubit fluorimeter (Thermo-Fisher). Evenly diluted product was used for a second round of amplification with Illumina linkers and barcodes with the following PCR conditions: 1 cycle 95°C for 10 min. 1 cycle 95°C for 15sec, 1 cycle of 60°C for 30 sec, 14 cycles of 72 °C for 1 minute followed by a 72 °C extension for 3 minutes. Products were quantified on a Qubit fluorimeter and stored at -20 C. All samples were run on a Fragment Analyzer (Advanced Analytics, Ames, IA) and amplicon regions and expected sizes confirmed. Samples were then pooled in equal amounts according to product concentration. The pooled products were then size selected on a 2% agarose E-gel (Life Technologies) and extracted from the isolated gel slice with Qiagen gel extraction kit (Qiagen). Cleaned size selected products were run on an Agilent Bioanalyzer to confirm appropriate profile and determination of average size. The final library pool was spiked with 10% non-indexed PhiX control library (Illumina®) and sequenced using Illumina® MiSeq® V3 Bulk system. The libraries were sequenced from both ends of the molecules to a total read length of 300 nt from each end. High-quality (> 25) sequence data (FASTQ) were analyzed with QIIME 1.9.0[54]. Quality control consisted of depleting or removing barcodes, primers, and short sequences (< 237 bp), sequences with ambiguous base calls, and sequences with homopolymer runs exceeding 6 bp. After removal of singletons, OTUs were classified using closed reference picking with the Ribosomal Database Project (RDP) at 97% similarity. β-diversity (weighted and unweighted UniFrac distances) were computed at an even sampling depth of 14,201 sequences per sample based on α-diversity (Chao1) rarefaction plots (see Figure, Supplemental Digital Content 3, Rarefaction α- diversity plot, http://links.lww.com/MSS/B106).

SCFA analysis

SCFAs were analyzed as described previously by Panasevich et al.(22). Briefly, fresh fecal samples were acidified in 6.25% meta-phosphoric acid solution and stored at −20°C. SCFA concentrations were determined by gas chromatography (Hewlett-Packard 5890A Series II) and a glass column (180 cm × 4 mm i.d.), packed with 10% SP-1200/1% H_3PO_4 on 80/100 + mesh Chromosorb WAW (Supelco, Inc.). Nitrogen was the carrier gas with a flow rate of 75 mL/min. Oven, detector, and injector temperatures were 125°C, 175°C, and 180°C, respectively. Acetic, n-butyric, and propionic acid solutions (Sigma-Aldrich) were used as standards.

Functional gene quantification

Isolated bacterial DNA was assessed for the relative abundance of butyryl CoA: acetate CoA transferase gene (BCoAT) and methylmalonyl CoA (mmDA) content by qPCR as described by Louis and Flint (2007) and Reichardt (2014), repectively (23, 24). Briefly, real-time PCR experiments were performed with SYBR Green Master Mix (Applied Biosystems) in a total volume of 20 μL. BCoAT and mmDA genes were quantified in parallel with a universal 16S rRNA gene probe (see Table, Supplemental Digital Content 4, qPCR target genes and primer sequences, http://links.lww.com/MSS/B107).

Statistical Analysis

Gut Microbiota Diversity and Taxonomy Evaluation

β-diversity was calculated using QIIME version 1.9.0 (25) at an even sample depth of 14,038 sequences per sample, visualized using *EMPeror (26),* and analyzed by permutational multivariate analysis of variance (PERMANOVA). Genera level taxonomy assignment was generated using RDP database through QIIME and was used as the primary outcome variable in subsequent factor analysis analyzing changes in gut microbiome. Pre-exercise (E0) to post-

exercise (E6) changes in SCFA concentrations, SCFA producing genes, body composition, and VO_{2max} were analyzed using a 2 x 2 repeated measures analysis of variance (ANOVA) with BMI (Lean, Obese) and Exercise [Time] (E0 - E6) as independent factors. Due to non-universal adherence to washout period guidelines, Post exercise (E6) to Washout (W6) changes in SCFA concentrations, relative SCFA producing genes, body composition, and VO_{2max} were completed using a separate 2 x 2 repeated measures ANOVA with BMI and Washout [Time] (E6-W6) as independent variables.

Random Forest Analysis and Boruta Feature Selection

To determine whether exercise training induced orthogonal shifts in the gut microbiota based on obesity status, Random Forest (RF) analysis was performed. The training set utilized pre- to post-exercise change in bacterial genera (Δ E0-E6 expressed as % of total bacteria) with BMI status as the grouping variable. RF was also performed with the change in bacterial genera during the washout (Δ E6-W6 % of total bacteria) as the independent training set. The cross-validation error rates of correctly predicted groupings were averaged across BMI groups compared to the baseline error rates that would be achieved by randomly assigning to either grouping variable by Matthews Correlation Coefficient (see Figure, Supplemental Digital Content 5, Random Forest classification, http://links.lww.com/MSS/B108). Next, Boruta feature selection was implemented to determine which shifts in bacterial taxa best discriminated between lean and obese groups. Boruta selection uses RF to iteratively compare importance of independent variables with that of pseudo-random (shadow) attributes(27). Variables 'confirmed' by Boruta feature selection had significantly higher RF importance scores than 'shadow' attributes and could therefore delineate between lean and obese groups. Analysis was performed on *R random forest package* with 1000 trees and all other default values.

Principle Component Factor Reduction

To determine if exercise caused physiologically relevant changes in gut microbiota composition, principle component factor analysis (PCA) was completed on the change scores of bacterial genera (ΔE0-E6) that paralleled similar changes in SCFA (fecal butyrate or BCoAT; or cardiorespiratory fitness (VO_{2max}) within each BMI group. First, the analyses revealed that the change in seven bacterial genera paralleled change in butyrate or BCoAT production in the lean participants and ten genera paralleling change in VO_{2max} in the obese participants via *Spearman Rho* correlations. Next, retracted components explaining the overall change in these bacteria were calculated by PCA. This factor equation was then applied (unbiased) to the washout change scores of the same genera (ΔE6-W6). Resulting factors were then compared to each other and to outcome variables through Pearson r correlation analysis. Loading variables with small loading coefficients (< 0.4) were suppressed for all PCA analysis. Significance was set *a priori* at $\alpha \leq 0.05$ for analyses.

RESULTS

Exercise training improved body composition and VO_{2max}, which were reversed after returning to sedentary activity.

Exercise training increased total lean body mass ($p < 0.01$, **Fig. 1A**), decreased body fat percentage ($p < 0.01$, **Fig 1B**), and increased bone mineral density ($p < 0.05$, **Fig 1C**) in both lean and obese subjects. Exercise also led to a marked improvement in cardiorespiratory fitness as measured by relative VO_{2max} in both lean and obese groups ($p < 0.05$, **Fig 1D).** After a return to sedentary activity for six weeks (W6), lean mass and body fat changes that occurred in response to training were reversed towards baseline values in both lean and obese participants ($p < 0.05$; **Figure 1A-B);** while BMD remained higher versus baseline (**Figure 1C**). VO_{2max} was not measured at W6.

Exercise induces orthogonal shifts in the gut microbiome dependent on BMI status

β-diversity analysis revealed that gut microbiota composition was different between lean and obese individuals at baseline (E0 PERMANOVA p = 0.034; **Fig. 2A**). After exercise training, there was no difference between the two groups (E6, PERMANOVA p = 0.31; **Fig. 2B**) and remained not significantly different after the washout period (W6, PERMANOVA p = 0.27; **Fig 2C**). To further investigate whether exercise-training initiated orthogonal shifts in gut microbiota communities' dependent on obesity status, RF analysis using pre- to post-exercise change scores of bacterial genera was implemented. Change scores of genera could be adequately trained to predict obesity status (Cross Validation Error Rate = 19.35 $\pm$ 3.32, $p < 0.05$ versus Baseline Entry; see Figure A, Supplemental Digital Content 5, Random Forest classification, http://links.lww.com/MSS/B108)**.** Next, RF was applied to change scores of bacterial genera from post-exercise (E6) to washout (W6) and again, could be used to predict obesity status (Cross Validation Error Rate=18.21 $\pm$ 13.35, $p<0.05$ versus Baseline Entry; see Figure B, Supplemental Digital Content 5, Random Forest classification, http://links.lww.com/MSS/B108). Bacterial taxa that shifted differentially in response to exercise as a result of obesity status (confirmed by Boruta feature selection) are shown in **Figure 2D** (Exercise) and **Figure 2E** (Washout). Notably, changes in three bacterial genera (*Collinsella spp., Faecalibacterium spp. and Lachnospira spp.)* were strong predictors of BMI status across both RF analyses and responded in a contrasting manner as a result of exercise training (E0-E6; **Figure 2D**) compared to washout (E6-W6; **Figure 2E**). No differences in α-diversity of the microbiota were observed between lean and obese participants at baseline (see Figure, **Supplemental Digital Content 3**, **Rarefaction α- diversity plot, http://links.lww.com/MSS/B106)**; $p > 0.05$) or at any of remaining collection time points ($p > 0.05$; data not shown).

Exercise training increased fecal SCFA concentrations and gut microbiome SCFA-producing capacity depending on BMI status

We investigated the effects of exercise training on fecal SCFA concentrations and genes within the gut microbiota important for SCFA production and observed that aerobic exercise increased fecal concentrations of the three most abundant SCFAs: acetate [C2], propionate [C3] and butyrate [C4]. This effect appeared to be dependent on BMI status, as the change in acetate and butyrate were observed primarily in the lean participants (Exercise x BMI $p < 0.05$; **Fig. 3Ai and Figure 3Aiii**). Changes in SCFAs were observed concomitant to an exercise-induced increase in the relative abundance of the butyrate-regulating gene BCoAT and the propionate-regulating gene mmDA ($p < 0.05$, respectively; **Fig. 3Bi-ii**). At W6, fecal acetate concentrations remained elevated ($p > 0.05$), while propionate and butyrate declined towards baseline levels ($p < 0.05$). In the obese group, concentrations of all three SCFAs did not change from E6 to W6 ($p > 0.05$; **Fig 3Ai-iii).** However, the washout period was accompanied by a reduction in SCFA-producing capacity of the gut microbiome, as depicted by E6 to W6 reduction in relative abundance of BCoAT and mmdA ($p < 0.05$; **Fig. 3Bi-ii**).

In obese individuals, we observed greater relative BCoAT compared to lean individuals ($p < 0.01$; **Fig. 3Bi**). The differences in baseline BCoAT levels, however, were not corroborated by differences in fecal SCFA concentrations between BMI categories at baseline (**Fig. 3Ai-iii**). Interestingly, and contrary to the notion that the SCFA-producing capacity of the gut microbiota may be detrimental to obese individuals, relative concentrations of SCFAs and BCoAT were associated with lower body fat (%), higher lean mass (%) and higher VO_{2max} within the obese group at baseline (see Figures A-D, **Supplemental Digital Content 6**, **SCFAs and functional genes versus baseline measures, http://links.lww.com/MSS/B109;** $p<0.05$, respectively).

Exercise training increases butyrate-regulating bacterial taxa that strongly associate with parallel shifts in body composition in lean individuals

Despite observing clear overall shifts in community composition, few individual taxa were consistently altered by exercise training across all individuals. In light of this apparent individualized response, we focused on whether functional groups of bacteria (rather than individual taxa), changed as a result of physical activity. First, we examined whether there existed a '*SCFA-regulating bacterial group'* that was affected by exercise training in lean participants only, as SCFA concentrations in obese participants were altered to a lesser degree by exercise training compared to lean participants. Using Spearman rank correlations, we observed that the change from E0 to E6 of five bacterial genera, *Roseburia spp., Lachnospira spp., Clostridiales spp., Faecalibacterium spp.,* and *f Lachnospiraceae unclass.,* positively correlated with changes in butyrate and/or BCoAT, while changes in *Bacteroides spp.* and *Rikenella spp.* negatively correlated with changes in butyrate and/or BCoAT ($p < 0.05$ for both analyses; **Figure 4A**). Using principle components analysis, the relative changes in these selected taxa were then modeled into one factor, which explained 61.2% of the total variance in how these genera changed as a result of exercise training ($EXERCISE_{LN\_Factor1}$; **Figure 4B**). Next, we correlated this resulting factor against changes in body composition and VO_{2max} and report that exercise-induced changes in *'butyrate-regulating bacterial group'*, along with changes in SCFA concentrations and BCoAT levels, were significantly related to changes in body composition (e.g., increases in lean mass and reductions in fat mass) (**Figure 4C)**. Moreover, the five bacteria genera that were positively correlated to butyrate production were modeled into one factor termed *'Butyrate Producers'*. These bacterial genera (*Clostridiales spp, Lachnospira spp, Roseburia spp. f_Lachnospiraceae unclass and Faecalibacterium spp.*), which together consisted of over 30% of the total representative genera, increased in abundance with exercise training

irrespective of BMI ($p < 0.05$), and then decreased after a return to sedentary activity ($p < 0.05$; **Figure 4D**). Within the obese group, we observed fewer relationships between genera changes and parallel shifts in SCFAs. Nevertheless, we observed a functional group of genera (10) that paralleled changes in cardiorespiratory fitness (see Figure, **Supplemental Digital Content 7**, **Obese microbiota changes versus $VO_{2\ max}$**, http://links.lww.com/MSS/B110).

A return to sedentary activity for six weeks led to a BMI dependent reversion in gut microbiome composition

Lastly, we investigated whether a return to sedentary activity (E6-W6) would reverse the gut microbiota changes observed after exercise training. To accomplish this, we analyzed the effects of exercise training (ΔE0-E6) and return to sedentary activity (ΔE6-W6) on the bacterial genera within the BMI dependent bacterial groups depicted in **Figure 4 (LN)** and Figure, **Supplemental Digital Content 7**, **Obese microbiota changes versus $VO_{2\ max}$**, http://links.lww.com/MSS/B110 **(OB)**. First, our results reveal that the relationship between genera changes that occurred from ΔE0-E6 were inversely related to the genera changes that occurred from ΔE6-W6. In fact, five out of the seven bacterial genera in the lean bacterial clade and eight out of the ten genera in the obese bacterial clade were inversely regulated by PA status (**Figure 5Ai. and Bi.**). We then used the component factor equation that most thoroughly explained the change in bacterial genera from E0-E6 ($EXERCISE_{factor1}$) to calculate a factor explaining genera changes that occurred during E6-W6 ($WASH_{factor1}$). Using this unbiased predictive model, we found that the calculated factor component from the washout period $WASH_{factor1}$ was inversely correlated to that of the factor component from exercise period $EXERCISE_{factor1}$ within both BMI categories (Lean $r = -0.831$, $p < 0.01$, **Fig 5Aii.** Obese $r = -0.630$, $p < 0.05$; **Fig. 5Bii.**), thus further validating that gut microbiota composition is dependent upon PA status.

DISCUSSION

In this study, we provide evidence that six weeks of aerobic exercise training alters the gut microbiota and microbial-derived SCFAs in previously sedentary lean and obese adults without any changes to dietary patterns. At baseline, obese individuals had different gut microbiota compositions from lean individuals, and the microbiotas of the two groups responded differently to exercise training. Additionally, we demonstrate that exercise-induced modulations of the gut microbiota and SCFAs were strongly associated with changes in body composition in lean participants and VO_{2max} in obese participants, highlighting a role of the gut microbiota in regulating physiological adaptations to exercise training. Lastly, we report that gut microbiome changes that occurred after a return to sedentary activity were inversely related to the modifications that occurred in response to exercise training, providing further evidence that physical activity status contributes significantly to the composition of the human gut microbiota.

This study revealed that compositional and functional microbiome changes due to exercise participation (or a return to sedentary activity) were dependent on BMI. This was indicated by obesity-dependent differences in baseline gut microbiota composition that were not maintained after six weeks of exercise. Further highlighting the BMI-dependent response to training, exercise-induced shifts in SCFA-producing taxa (*Faecalibacterium* spp. and *Lachnospira* spp.) and genetic machinery (BCoAT) were more substantial in lean versus obese participants.

Exercise-induced increases in fecal SCFA concentrations observed in this study corroborate data presented in rodent models (8), as well as a previous cross-sectional study in humans relating fecal butyrate concentrations to VO_{2max} and muscle turnover (12). The mechanism(s) responsible for exercise-induced increases in fecal SCFA concentration are not yet clear, but could involve

increased production of SCFA through endogenous metabolic input (e.g. lactate), increased mixing of intestinal contents and bacterial fermentation of dietary fibers, increased anaerobic fermentation due to colonic oxygen saturation or pH changes, or reduced intestinal utilization and uptake of SCFA (28). Exercise-induced increases in fecal SCFA may also be augmented by shifts in the gut microbiota's functional capacity to produce SCFA. Of the genes within the gut microbiota involved in SCFA production, Butyryl CoA: Acetate CoA transferase (BCoAT) is one of two known butyrate-producing genes that contributes significantly to butyrate levels (29). Functionally, BCoAT transfers a co-enzyme A group from butyrl CoA to acetate, which results in the formation of acetyl CoA and butyrate, two substrates utilized by tissues for macromolecule synthesis or energy production (30). Methylmalonyl-CoA decarboxylase (mmdA), meanwhile, is an integral gene in the succinate pathway, one of three propionate regulating pathways highly conserved within the mammalian gut microbiota (24). In light of the prominent roles of these genes in SCFA production and their strong association with SCFA-producing bacteria, we hypothesize that exercise-induced increases in these functional genes may partially explain the parallel rise in fecal SCFAs.Moreover, it is possible that these shifts in SCFA genetic machinery may augment responses to other environmental cues within the gut, including increased potential for dietary fiber fermentation, ultimately leading to increased SCFA production (31).

Exercise-induced increases in fecal BCoAT and SCFA levels also strongly paralleled improvements in body composition, including most notably, increases in lean mass . The mechanisms behind such an association are unclear, but many possible explanations exist. SCFAs can improve skeletal muscle insulin sensitivity (32), reduce inflammation (33), and regulate satiety (34), all of which may contribute to the improvements in body composition observed in this study. Additionally, SCFAs are also energy substrates for numerous tissue types,

including colon (35), adipose (36), and muscle (32), indicating that SCFAs can contribute to enhanced energy harvest from the diet, ultimately leading to enhanced tissue growth, including skeletal muscle. Considering the findings presented here and previous cross-sectional studies (12, 13), we postulate that an exercise-induced increase towards a greater SCFA-producing capacity may represent a beneficial adaptation, ultimately contributing to enhanced replenishment of expended calories and improvements in body composition. The SCFA-producing capacity of the gut microbiota also returned to pre-training levels after the washout period, as indicated by a return of fecal BCoAT and mmDA levels (within the lean and obese groups) and butyrate-producing bacteria taxa (within the lean group) toward baseline values. In light of these findings, we postulate that exercise-induced shifts in the metabolic capacity of the gut microbiome may be transient and likely dependent on repeated exercise stimuli.

This study also identified differences in the baseline microbiome characteristics and SCFA producing capabilities between lean and obese individuals. Most notably, obese participants displayed nearly double the baseline levels of BCoAT compared to lean counterparts. The possible role of BCoAT and SCFAs in energy harvest during obesity was first described in a seminal study by Turnbaugh et al.(14), who showed that a fecal transplant from obese individuals into germ-free (GF) mice led to increased gut SCFA concentrations and an obese phenotype in the recipient mice. In humans, successful weight loss through gastric bypass surgery was accompanied by reduced levels of BCoAT (37). However, contrary to previous studies, we observed no differences in the concentrations of any SCFAs between lean and obese individuals. Moreover, and conflicting with the notion that SCFAs lead to aberrant body composition phenotypes during obesity, this study indicates that within the obese participants, baseline fecal butyrate concentrations were strongly associated with higher lean mass, reduced body fat, and higher cardiorespiratory fitness (VO_{2max}), while BCoAT levels were associated

with higher lean mass. Therefore, these data challenge the assumption that the gut microbiome's potential to produce SCFAs is a biomarker for a maladaptive obese phenotype. Rather, it appears that these factors are indicative of healthier body composition and enhanced cardiorespiratory fitness during obesity.

Exercise-induced shifts in the gut microbiota and SCFAs may have implications for diseases of the gut. Physical activity has been shown to reduce risk of colorectal cancer and irritable bowel disease (IBD) (38). Butyrate is a primary fuel source for colonocytes (39), is required for maintaining gut barrier function (40), and is a critical metabolite regulating the anti-inflammatory/regulatory phenotypes of gut-resident immune cells (41). Interestingly, genera that positively associated with exercise-induced increases in butyrate concentrations (*Faecalibacterium spp., Roseburia spp., Lachnospira spp., f_Lachnospiraceae,* and *Clostridiales spp*.), are well documented butyrate producers (31, 42), and have been associated with notable physiological outcomes *in vivo*. In particular, *Faecalibacterium spp.*_has been shown to induce local and systemic anti-inflammatory effects and may be protective against bowel diseases, including ulcerative colitis (43, 44). Thus, we postulate that exercise-induced shifts in SCFAs, butyrate-producing taxa and metabolic capacity to produce SCFA may have the potential to prevent the occurrence or offset the symptomology of IBD. Future studies are needed to address these hypotheses more closely.

There are limitations to this study. Seven participants did not adhere to washout guidelines and were excluded from analysis. We also did not include VO_2 max at the end of the study due to predicted subject burden of an extended exercise training study. We did, however, assess washout body composition to verify that physiological parameters reverted to pre-exercise levels after a return to sedentary activity.

In summary, these data indicate for the first time that exercise training can significantly modulate the composition and metabolic capacity of the human gut microbiota. Exercise-induced changes in the gut microbiota are valuable prognostic features that are integral to understanding the overall physiological response to exercise training in humans.

Acknowledgements

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Conflict of Interest Statement

Authors have no professional relationships with companies or manufacturers who will benefit from the results of the present study. Results of the present study do not constitute endorsement by ACSM. Results of the study are presented clearly, honestly and without fabrication, falsification, or inappropriate data manipulation.

REFERENCES

1. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2013. doi: 10.1038/nature12820. PubMed PMID: 24336217.

2. Cani PD. Gut microbiota and obesity: lessons from the microbiome. Briefings in functional genomics. 2013;12(4):381-7. Epub 2013/04/26. doi: 10.1093/bfgp/elt014. PubMed PMID: 23616309.

3. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(26):11971-5. Epub 2010/06/23. doi: 10.1073/pnas.1002601107. PubMed PMID: 20566857; PubMed Central PMCID: PMC2900693.

4. Allen JM, Berg Miller ME, Pence BD, Whitlock K, Nehra V, Gaskins HR, et al. Voluntary and forced exercise differentially alters the gut microbiome in C57BL/6J mice. J Appl Physiol (1985). 2015;118(8):1059-66. doi: 10.1152/japplphysiol.01077.2014. PubMed PMID: 25678701.

5. Choi JJ, Eum SY, Rampersaud E, Daunert S, Abreu MT, Toborek M. Exercise attenuates PCB-induced changes in the mouse gut microbiome. Environmental health perspectives. 2013;121(6):725-30. doi: 10.1289/ehp.1306534. PubMed PMID: 23632211; PubMed Central PMCID: PMC3672930.

6. Kang SS, Jeraldo PR, Kurti A, Miller ME, Cook MD, Whitlock K, et al. Diet and exercise orthogonally alter the gut microbiome and reveal independent associations with anxiety and cognition. Mol Neurodegener. 2014;9:36. doi: 10.1186/1750-1326-9-36. PubMed PMID: 25217888; PubMed Central PMCID: PMCPMC4168696.

7. Evans CC, LePard KJ, Kwak JW, Stancukas MC, Laskowski S, Dougherty J, et al. Exercise prevents weight gain and alters the gut microbiota in a mouse model of high fat diet-induced obesity. PloS one. 2014;9(3):e92193. Epub 2014/03/29. doi: 10.1371/journal.pone.0092193. PubMed PMID: 24670791; PubMed Central PMCID: PMC3966766.

8. Matsumoto M, Inoue R, Tsukahara T, Ushida K, Chiji H, Matsubara N, et al. Voluntary running exercise alters microbiota composition and increases n-butyrate concentration in the rat cecum. Biosci Biotechnol Biochem. 2008;72(2):572-6. doi: 10.1271/bbb.70474. PubMed PMID: 18256465.

9. Kaczmarczyk MM, Miller MJ, Freund GG. The health benefits of dietary fiber: beyond the usual suspects of type 2 diabetes mellitus, cardiovascular disease and colon cancer. Metabolism: clinical and experimental. 2012;61(8):1058-66. doi: 10.1016/j.metabol.2012.01.017. PubMed PMID: 22401879; PubMed Central PMCID: PMC3399949.

10. Watterson KR, Hudson BD, Ulven T, Milligan G. Treatment of type 2 diabetes by free Fatty Acid receptor agonists. Frontiers in endocrinology. 2014;5:137. Epub 2014/09/16. doi: 10.3389/fendo.2014.00137. PubMed PMID: 25221541; PubMed Central PMCID: PMC4147718.

11. De Vadder F, Kovatcheva-Datchary P, Goncalves D, Vinera J, Zitoun C, Duchampt A, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell. 2014;156(1-2):84-96. Epub 2014/01/15. doi: 10.1016/j.cell.2013.12.016. PubMed PMID: 24412651.

12. Estaki M, Pither J, Baumeister P, Little JP, Gill SK, Ghosh S, et al. Cardiorespiratory fitness as a predictor of intestinal microbial diversity and distinct metagenomic functions. Microbiome. 2016;4(1):42. doi: 10.1186/s40168-016-0189-7. PubMed PMID: 27502158; PubMed Central PMCID: PMCPMC4976518.

13. Barton W, Penney NC, Cronin O, Garcia-Perez I, Molloy MG, Holmes E, et al. The microbiome of professional athletes differs from that of more sedentary subjects in composition and particularly at the functional metabolic level. Gut. 2017. doi: 10.1136/gutjnl-2016-313627. PubMed PMID: 28360096.

14. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444(7122):1027-31. doi: 10.1038/nature05414. PubMed PMID: 17183312.

15. McPhee JB, Schertzer JD. Immunometabolism of obesity and diabetes: microbiota link compartmentalized immunity in the gut to metabolic tissue inflammation. Clinical science. 2015;129(12):1083-96. Epub 2015/10/16. doi: 10.1042/CS20150431. PubMed PMID: 26464517.

16. Woods JA, Vieira VJ, Keylock KT. Exercise, inflammation, and innate immunity. Immunol Allergy Clin North Am. 2009;29(2):381-93. Epub 2009/04/25. doi: S0889-8561(09)00012-5 [pii]

10.1016/j.iac.2009.02.011. PubMed PMID: 19389588.

17. Hammer RL, Barrier CA, Roundy ES, Bradford JM, Fisher AG. Calorie-restricted low-fat diet and exercise in obese women. The American journal of clinical nutrition. 1989;49(1):77-85. PubMed PMID: 2912014.

18. Campbell SC, Wisniewski PJ, Noji M, McGuinness LR, Haggblom MM, Lightfoot SA, et al. The Effect of Diet and Exercise on Intestinal Integrity and Microbial Diversity in Mice. PloS one. 2016;11(3):e0150502. doi: 10.1371/journal.pone.0150502. PubMed PMID: 26954359; PubMed Central PMCID: PMC4783017.

19. Goldberg Y, Boaz M, Matas Z, Goldberg I, Shargorodsky M. Weight loss induced by nutritional and exercise intervention decreases arterial stiffness in obese subjects. Clinical nutrition. 2009;28(1):21-5. Epub 2008/11/21. doi: 10.1016/j.clnu.2008.10.001. PubMed PMID: 19019502.

20. Pollock ML, Foster C, Schmidt D, Hellman C, Linnerud AC, Ward A. Comparative analysis of physiologic responses to three different maximal graded exercise test protocols in healthy women. Am Heart J. 1982;103(3):363-73. PubMed PMID: 7064770.

21. Muturi EJ, Donthu RK, Fields CJ, Moise IK, Kim CH. Effect of pesticides on microbial communities in container aquatic habitats. Scientific reports. 2017;7:44565. doi: 10.1038/srep44565. PubMed PMID: 28300212; PubMed Central PMCID: PMC5353589.

22. Panasevich MR, Allen JM, Wallig MA, Woods JA, Dilger RN. Moderately Fermentable Potato Fiber Attenuates Signs and Inflammation Associated with Experimental Colitis in Mice. The Journal of nutrition. 2015;145(12):2781-8. Epub 2015/10/23. doi: 10.3945/jn.115.218578. PubMed PMID: 26491118.

23. Louis P, Flint HJ. Development of a semiquantitative degenerate real-time pcr-based assay for estimation of numbers of butyryl-coenzyme A (CoA) CoA transferase genes in complex bacterial samples. Applied and environmental microbiology. 2007;73(6):2009-12. Epub 2007/01/30. doi: 10.1128/AEM.02561-06. PubMed PMID: 17259367; PubMed Central PMCID: PMC1828812.

24. Reichardt N, Duncan SH, Young P, Belenguer A, McWilliam Leitch C, Scott KP, et al. Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. The ISME journal. 2014;8(6):1323-35. Epub 2014/02/21. doi: 10.1038/ismej.2014.14. PubMed PMID: 24553467; PubMed Central PMCID: PMC4030238.

25. Kuczynski J, Stombaugh J, Walters WA, Gonzalez A, Caporaso JG, Knight R. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Current protocols in microbiology. 2012;Chapter 1:Unit 1E 5. Epub 2012/11/28. doi: 10.1002/9780471729259.mc01e05s27. PubMed PMID: 23184592.

26. Vazquez-Baeza Y, Pirrung M, Gonzalez A, Knight R. EMPeror: a tool for visualizing high-throughput microbial community data. GigaScience. 2013;2(1):16. doi: 10.1186/2047-217X-2-16. PubMed PMID: 24280061; PubMed Central PMCID: PMC4076506.

27. Kursa MB. Robustness of Random Forest-based gene selection methods. BMC bioinformatics. 2014;15:8. doi: 10.1186/1471-2105-15-8. PubMed PMID: 24410865; PubMed Central PMCID: PMC3897925.

28. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. The Journal of physiology. 2009;587(Pt 17):4153-8. Epub 2009/06/06. doi: 10.1113/jphysiol.2009.174136. PubMed PMID: 19491241; PubMed Central PMCID: PMC2754355.

29. Louis P, McCrae SI, Charrier C, Flint HJ. Organization of butyrate synthetic genes in human colonic bacteria: phylogenetic conservation and horizontal gene transfer. FEMS microbiology letters. 2007;269(2):240-7. Epub 2007/01/24. doi: 10.1111/j.1574-6968.2006.00629.x. PubMed PMID: 17241242.

30. Charrier C, Duncan GJ, Reid MD, Rucklidge GJ, Henderson D, Young P, et al. A novel class of CoA-transferase involved in short-chain fatty acid metabolism in butyrate-producing human colonic bacteria. Microbiology. 2006;152(Pt 1):179-85. Epub 2005/12/31. doi: 10.1099/mic.0.28412-0. PubMed PMID: 16385128.

31. Hippe B, Zwielehner J, Liszt K, Lassl C, Unger F, Haslberger AG. Quantification of butyryl CoA:acetate CoA-transferase genes reveals different butyrate production capacity in individuals according to diet and age. FEMS microbiology letters. 2011;316(2):130-5. Epub 2011/01/06. doi: 10.1111/j.1574-6968.2010.02197.x. PubMed PMID: 21204931.

32. Canfora EE, Jocken JW, Blaak EE. Short-chain fatty acids in control of body weight and insulin sensitivity. Nature reviews Endocrinology. 2015;11(10):577-91. Epub 2015/08/12. doi: 10.1038/nrendo.2015.128. PubMed PMID: 26260141.

33. Roelofsen H, Priebe MG, Vonk RJ. The interaction of short-chain fatty acids with adipose tissue: relevance for prevention of type 2 diabetes. Beneficial microbes. 2010;1(4):433-7. doi: 10.3920/BM2010.0028. PubMed PMID: 21831781.

34. Kaji I, Karaki S, Kuwahara A. Short-chain fatty acid receptor and its contribution to glucagon-like peptide-1 release. Digestion. 2014;89(1):31-6. Epub 2014/01/25. doi: 10.1159/000356211. PubMed PMID: 24458110.

35. Astbury SM, Corfe BM. Uptake and metabolism of the short-chain fatty acid butyrate, a critical review of the literature. Current drug metabolism. 2012;13(6):815-21. PubMed PMID: 22571479.

36. den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk TH, et al. Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity via a PPARgamma-Dependent Switch From Lipogenesis to Fat Oxidation. Diabetes. 2015;64(7):2398-408. Epub 2015/02/20. doi: 10.2337/db14-1213. PubMed PMID: 25695945.

37. Patrone V, Vajana E, Minuti A, Callegari ML, Federico A, Loguercio C, et al. Postoperative Changes in Fecal Bacterial Communities and Fermentation Products in Obese Patients Undergoing Bilio-Intestinal Bypass. Front Microbiol. 2016;7:200. doi: 10.3389/fmicb.2016.00200. PubMed PMID: 26941724; PubMed Central PMCID: PMCPMC4762995.

38. Wolin KY, Yan Y, Colditz GA, Lee IM. Physical activity and colon cancer prevention: a meta-analysis. British journal of cancer. 2009;100(4):611-6. doi: 10.1038/sj.bjc.6604917. PubMed PMID: 19209175; PubMed Central PMCID: PMC2653744.

39. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell metabolism. 2011;13(5):517-26. doi: 10.1016/j.cmet.2011.02.018. PubMed PMID: 21531334; PubMed Central PMCID: PMC3099420.

40. Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. The Journal of nutrition. 2009;139(9):1619-25. doi: 10.3945/jn.109.104638. PubMed PMID: 19625695; PubMed Central PMCID: PMC2728689.

41. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;504(7480):446-50. doi: 10.1038/nature12721. PubMed PMID: 24226770.

42. Louis P, Duncan SH, McCrae SI, Millar J, Jackson MS, Flint HJ. Restricted distribution of the butyrate kinase pathway among butyrate-producing bacteria from the human colon. J Bacteriol. 2004;186(7):2099-106. Epub 2004/03/19. PubMed PMID: 15028695; PubMed Central PMCID: PMC374397.

43. Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species Roseburia hominis and Faecalibacterium prausnitzii defines dysbiosis in patients with ulcerative colitis. Gut. 2014;63(8):1275-83. Epub 2013/09/12. doi: 10.1136/gutjnl-2013-304833. PubMed PMID: 24021287.

44. Qiu X, Zhang M, Yang X, Hong N, Yu C. Faecalibacterium prausnitzii upregulates regulatory T cells and anti-inflammatory cytokines in treating TNBS-induced colitis. J Crohns Colitis. 2013;7(11):e558-68. Epub 2013/05/07. doi: 10.1016/j.crohns.2013.04.002. PubMed PMID: 23643066.

Figure Legends

Figure 1. Changes from pre-exercise (E0) to post-exercise (E6) and washout (W6) for; **A)** lean mass % [upper right inset: absolute lean mass], **B)** body fat % (BF%) [upper right inset: absolute fat mass], **C)** bone mineral density (BMD) grams/cm^2 and **D)** relative maximal oxygen consumption (VO_{2max}) in ml/kg/min. * $p < 0.05$ versus E0 in respective group. N/A indicates that VO_{2max} tests were not administered at the washout time point.

Figure 2. A-C) Principle coordinates analysis (PCoA) of gut microbiota community composition (*Weighted Unifrac*) comparing Lean (LN) versus Obese (OB) groups at **A)** pre-exercise (E0), **B)** after exercise training for six weeks (E6) and, **C)** following a return to sedentary activity for six weeks (W6). PERMANOVA $p < 0.05$ deems significant difference between LN and OB microbiota communities, ns = not significantly different. **D-E)** Comparison of bacterial genera that changed orthogonally based on obesity status as a result of **D)** Exercise training (E0-E6) and **E)** Returning to sedentary activity washout (E6-W6); data are represented as Δ E0-E6 change (+ SEM). Genera represented were 'confirmed' by Boruta feature selection as satisfactory in describing obesity category.

Figure 3. A) Changes in fecal SCFA concentrations: **i.** acetate [C2], **ii.** propionate [C3] and **iii.** butyrate [C4] as a result of 6 weeks of exercise training (E0-E6) and returning to sedentary activity for six weeks (E6-W6, upper right graphs), **B)** changes in relative abundance of **i.** Butyryl CoA: acetate CoA transferase (BCoAT) and **ii.** methylmalonyl CoA decarboxylase (mmDA) as a result of six weeks of exercise training (E0-E6) and return to sedentary activity for six weeks (E6-W6, upper right graphs). *Denotes significant Time [Exercise or Washout] x Group [BMI] interaction effect; #denotes significant Exercise or Washout main effect; &denotes significant BMI main effect at $p < 0.05$.

Figure 4. A) Spearman correlations relating exercise-induced changes in seven bacterial genera to parallel changes in fecal concentrations of butyrate and relative abundance of butyryl CoA:acetate CoA transferase (BCoAT) within the LN group only. $EXERCISE_{LN\_Factor1}$ represents the component factor that most thoroughly explained the variance (61.2% of total) of the change in the highlighted bacterial genera. Spearman ρ, *p<0.05, **p<0.01 **B)** Loading coefficients for each selected taxa used in primary component factor explaining largest overall variance. $EXERCISE_{Ln\_FACTOR1}$ is representative of the factor component that most thoroughly explained the variance in change of these select bacteria genera (61.2%). **C)** Exercise-induced changes in butyrate-regulating taxa ($Exercise_{LN\_factor1}$), butyrate and BCoAT associate with parallel changes in body composition but not cardiorespiratory fitness within the LN participants (E0-E6), Pearson r, *p<0.05, **p<0.01. **D)** Indicates the effects of exercise training on the overall change in the five bacterial taxa *(Roseburia spp + Lachnospira spp. + f_Lachnospriaceae + Clostridiales spp. + Faecalibacterium spp*.) that positively associated with changes in butyrate or BCoAT concentrations, termed '*Butyrate Producers'*. # Main effect of Exercise (E0-E6) or Washout (E6-W6) at p<0.05.

Figure 5. Spearman correlations describing the relationship between the gut microbiota response to exercise-training (E0-E6) versus the gut microbiota responses to washout from exercise (E6-W6) regarding individual bacterial genera within: **Ai)** lean and **Bi)** obese 'functional clades'. **Aii – Bii)** Factor equations explaining most variance in bacterial change during exercise period among both BMI categories ($EXERCISE_{LN\_factor1}$ or $EXERCISE_{OB\_Factor1}$) were applied directly to those genera changes that occurred during the washout. The resulting factor component, termed $WASH_{LN\_factor1}$ or $WASH_{OB\_FACTOR1}$, was then compared with Pearson r correlations to the corresponding EXERCISE factor component.

Supplemental Digital Content

SDC 1.TIF Experimental Design. HRR= Heart Rate Reserve; Body Comp = Body Composition analysis through DEXA; VO_{2max} = Maximal Oxygen Uptake.

SDC 2.PDF Baseline dietary consumption A) Absolute (total intake/day) or **B)** Relative (% of total Kcal) macronutrient or fiber intake does not differ between lean and obese participants. Data are represented as Mean $\pm$ S.E.M. *Represents significant difference between Lean and Obese groups at $p < 0.05$.

SDC 3.TIF Rarefaction α- diversity plot. Rarefaction plot (Chao 1) at 14,201 sequences per sample (rarefaction depth).No statistical differences were observed between α-diversity of lean and obese participants at baseline or at any time point throughout the study $p>0.05$

SDC 4.PDF qPCR target genes and primer sequences. Forward and reverse primers (5'-3') used for relative gene quantification through qPCR.

SDC 5.TIF Random Forest classification Random Forest (RF) analysis was performed on the **A)** pre- to post-exercise change (E0-E6) and **B)** post-exercise to washout change (E6-W6) in bacterial genera as the training sets and with BMI status as the grouping variable. The cross-validation error rates (averaged across BMI groups) were compared to the baseline error rates that would be achieved by randomly assigning to either grouping variable through a Matthew's correlation coefficient.

SDC 6.TIF SCFAs and functional genes versus baseline measures A-C) Butyrate concentrations associate with **A)** higher lean mass, **B)** lower body fat and **C)** higher cardiorespiratory fitness (VO_{2max}) within obese participants at the baseline time point (E0). **D)** Butyrate regulating gene BCoAT also associates with higher relative VO_{2max} in obese participants at E0. Pearson correlation coefficients significant at $p < 0.05$, respectively.

SDC 7.TIF Obese microbiota changes versus VO_{2max} A) Spearman correlations relating changes in ten bacterial genera to parallel changes in cardiorespiratory fitness within the obese group only. Spearman ρ *p<0.05, ** p<0.01 **B)** Loading coefficients for each selected taxa used in primary component factor analysis. $EXERCISE_{OB\_FACTOR1}$ is representative of the factor component that most thoroughly explained the variance in change of these select bacteria genera (74.05%). **C)** $EXERCISE_{OB\_Factor1}$ was positively associated with changes in cardiorespiratory fitness within the obese group. Pearson r *p<0.05, **p<0.01

Figure 1

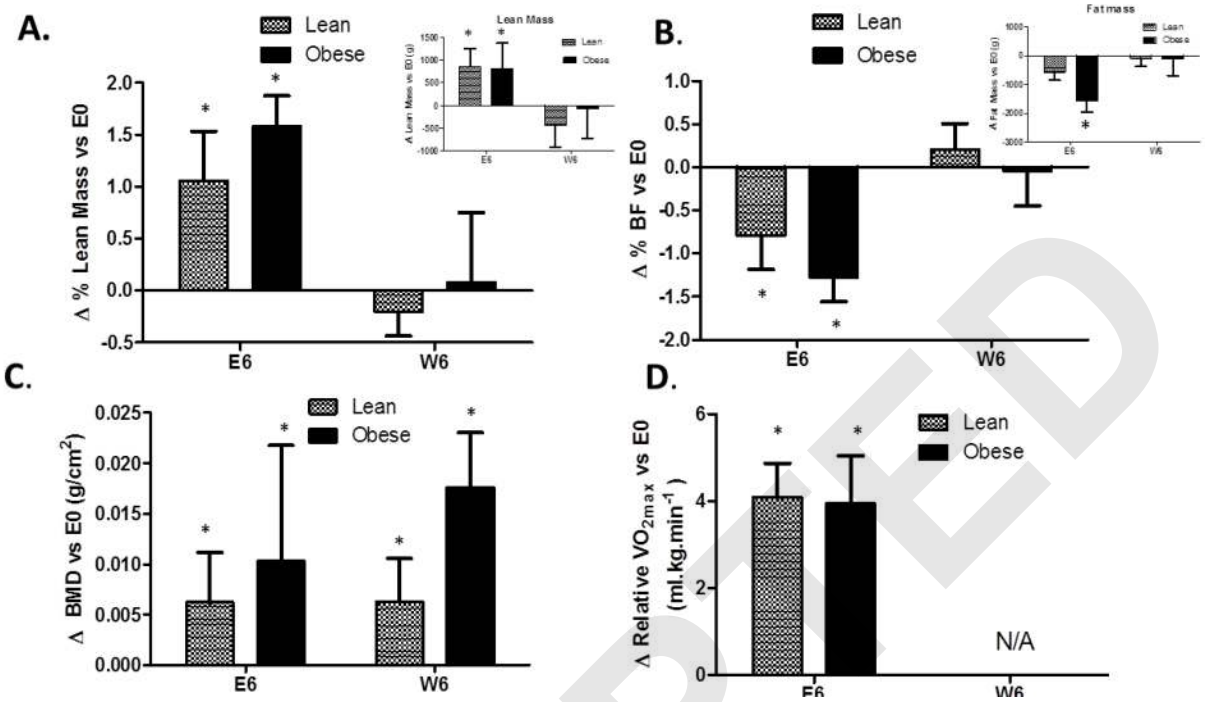


Figure 2

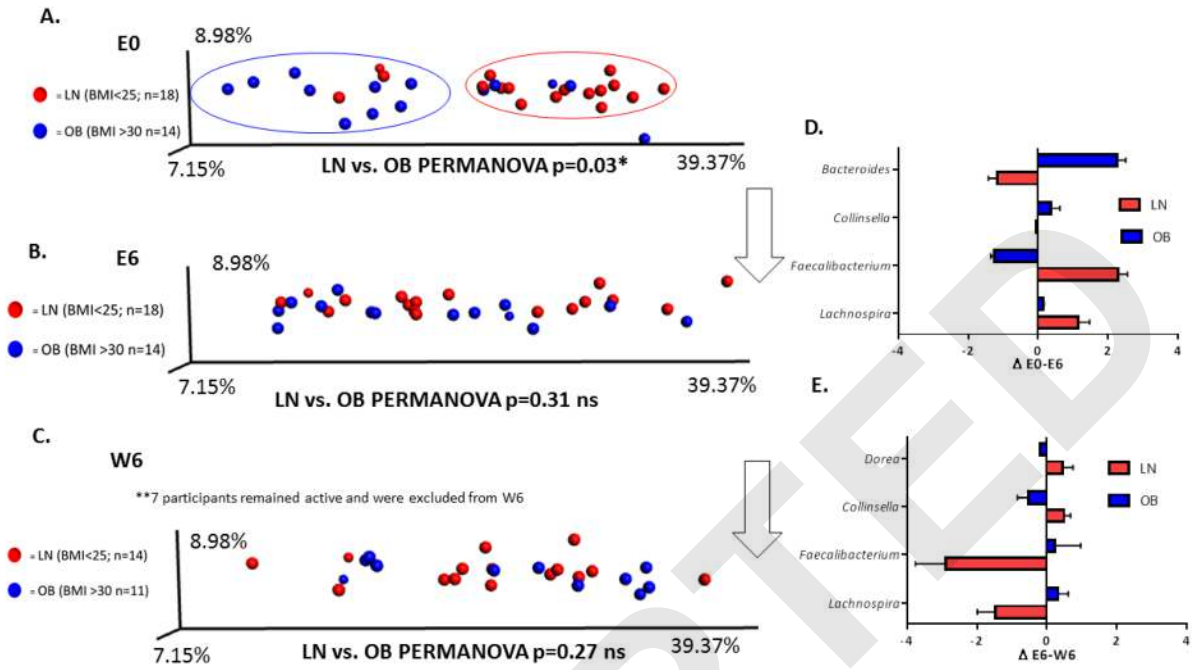


Figure 3

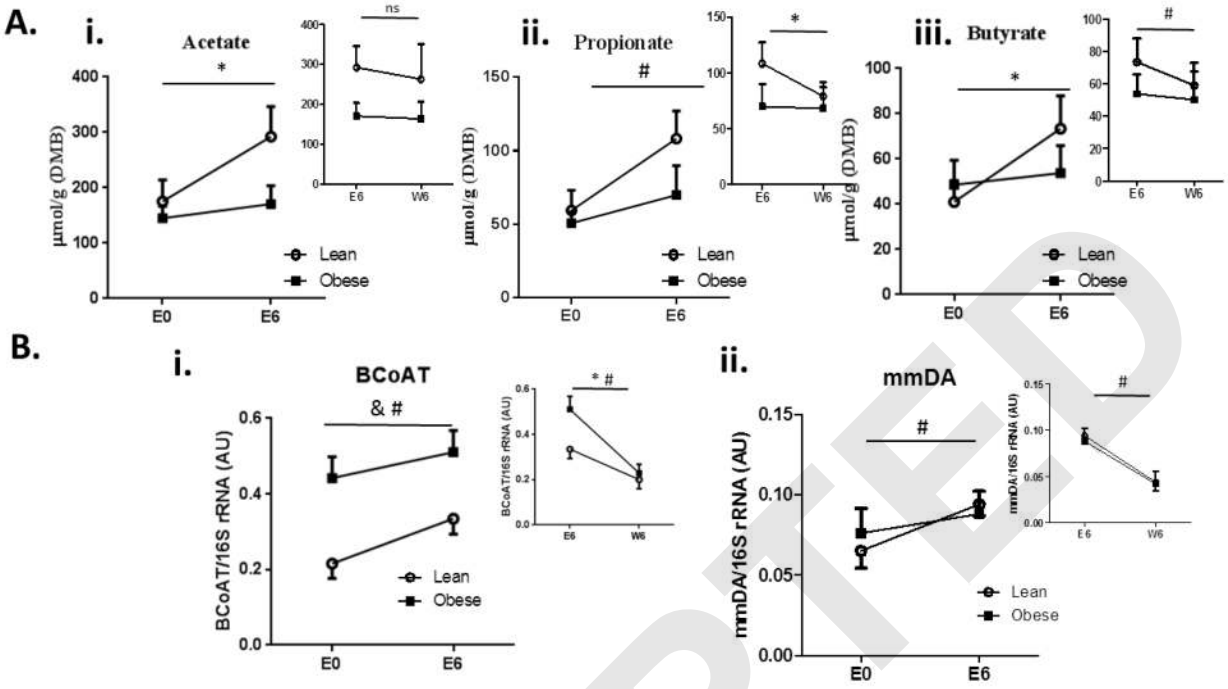


Figure 4

A.

Genus Δ E0 –E6	Butyrate Δ E0-E6 (Spearman ρ)	BCoAT Δ E0-E6 (Spearman ρ)
Bacteroides spp.	-0.690*	-0.299
Rikenella spp.	-0.613**	-0.424
Clostridiales spp	0.472*	0.188
Lachnospira spp.	0.541*	0.532*
f_Lachnospiraceae spp.	0.651**	0.426
Roseburia spp.	0.689**	0.508*
Faecalibacterium spp.	0.437*	0.462

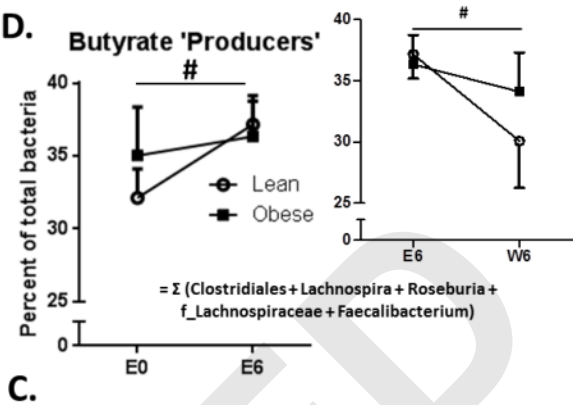
PCA factor reduction; LN group only

B.

Genus Δ E0 –E6	$Exercise_{LNFactor1}$ loading coefficient
Bacteroides spp.	-0.812
Rikenella spp.	-0.842
Clostridiales spp	0.558
Lachnospira spp.	0.540
f_Lachnospiraceae spp.	0.763
Roseburia spp.	0.796
Faecalibacterium spp.	0.412

$EXERCISE_{LN\_Factor1}$ explains 61.2% of variance

D.



C.

Pearson r

Δ E0 –E6	$EXERCISE_{LN\_Factor1}$	Butyrate	BCoAT
% Lean Mass	0.703**	0.873**	0.513*
% Body Fat	-0.574*	-0.499*	-0.46
VO_{2max}	0.187	0.269	0.351

FIGURE 5

Figure 5

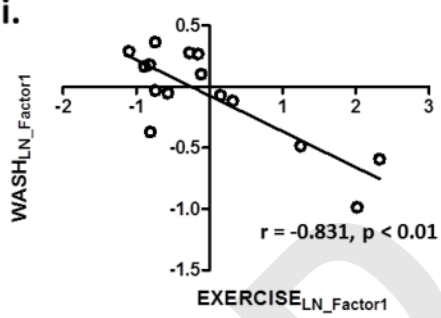
A.

i.

LN

Genera ($EXERCISE_{LN\_FACTOR1}$)	Δ E0-E6 vs. Δ E6-W6
Bacteroides spp.	-0.771**
Rikenella spp.	-0.754**
Lachnospira spp.	-0.688*
f_Lachnospiraceae spp.	-0.625*
Roseburia spp.	-0.657*
Faecalibacterium spp.	-0.532*
Clostridiales spp	-0.193

ii.



B.

i.

OB

Genera ($EXERCISE_{OB\_FACTOR1}$)	Δ E0-E6 vs. Δ E6-W6
Eggerthella spp.	-0.725*
f_Bifobacteriaceae spp.	-0.986**
Ruminococcus spp.	-0.698*
Coprobacillus spp.	-0.711*
Bifidobacterium spp.	-0.694*
Barnesiella spp.	-0.554*
Dorea spp.	-0.189
Lachnospira spp.	-0.746**
Paraprevotella	-0.245
Veillonella spp.	0.663*

ii.

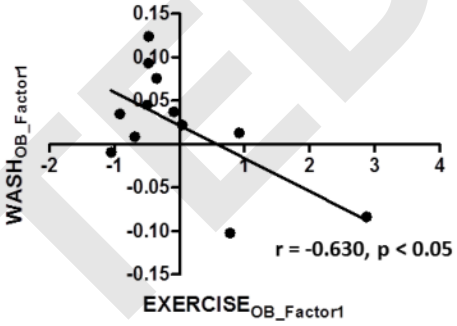
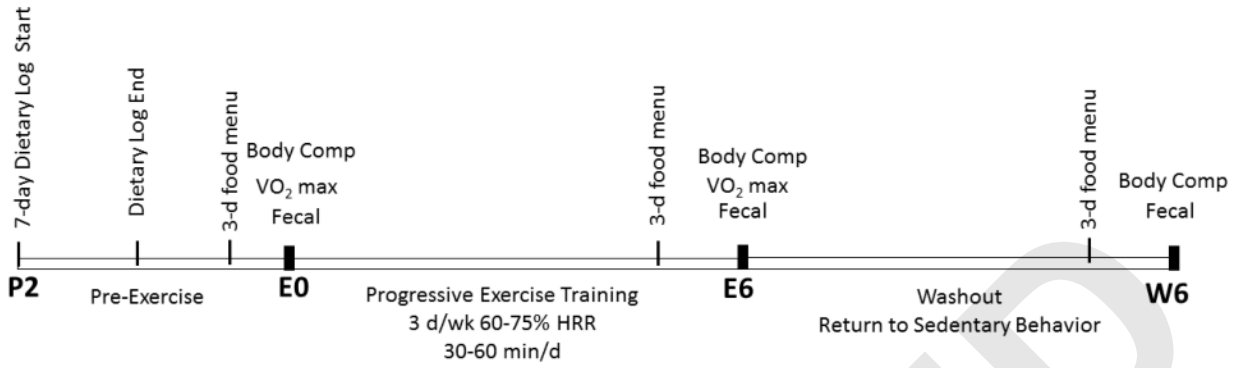


Table 1. Subject characteristics at baseline. Data are mean ± SEM. *Significant effect of BMI category at p<0.05.

	BMI Group	
	Lean (n=18); 9 female	**Obese (n=14); 11 female**
Age (yrs)	25.10 ± 6.52	31.14 ± 8.57
BMI (kg/m^2)	22.21 ± 2.76	35.71 ± 5.11*
Weight (kg)	58.92 ± 9.29	100.09 ± 23.60*
Body Fat %	26.04 ± 6.12	38.42 ± 4.98*
Lean Mass %	71.52 ± 6.18	59.42 ± 5.03*
Bone Density (g/cm^2)	1.11 ± 0.08	1.21 ± 0.12*
Absolute VO_{2Max}	2.51 ± 0.61	2.86 ± 0.89
Relative VO_{2Max}	38.99 ± 4.74	28.76 ± 6.01*



SDC 1. Experimental Design. HRR= Heart Rate Reserve; Body Comp = Body Composition analysis through DEXA; VO_{2max} = Maximal Oxygen Uptake.

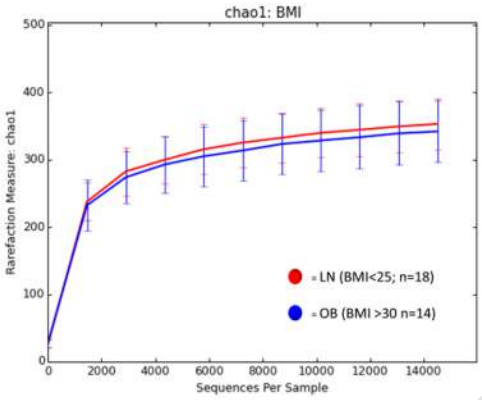
A.

Absolute Intake/Day	BMI Category	
	Lean (n=18)	Obese (n=14)
Energy (kcal)	2147.00 ± 141.00	2124.00 ± 101.40
Carbohydrate (g)	239.50 ± 13.65	230.50 ± 16.37
Protein (g)	98.99 ± 7.97	92.77 ± 4.42
Fat (g)	87.55 ± 7.62	86.91 ± 6.68
Total Dietary Fiber (g)	18.50 ± 1.46	17.78 ± 1.63
Soluble Dietary Fiber (g)	5.69 ± 0.42	5.77 ± 0.58
Insoluble Dietary Fiber (g)	12.73 ±1.16	11.89 ± 1.20

B.

Relative Intake/Day	BMI Group	
	Lean (n=18)	Obese (n=14)
Calories from Carbohydrate (% of total)	45.34 ± 1.38	42.97 ± 2.39
Calories from Protein (% of total)	18.92 ± 0.93	18.02 ± 0.89
Calories from Fat (%of total)	35.29 ± 1.22	35.86 ± 1.84
Dietary Fiber (g/1000 kcal)	9.07 ± 0.80	8.45 ± 0.66
Soluble Dietary Fiber (g/ 1000 kcal)	2.72 ± 0.18	2.70 ± 0.23
Insoluble Dietary Fiber (g/1000 kcal)	6.31 ± 0.68	5.70 ± 0.53

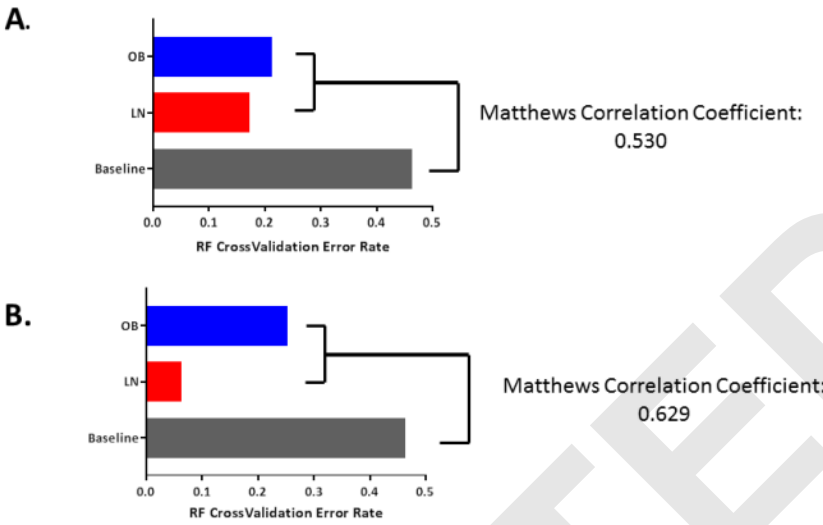
SDC 2. Baseline dietary consumption A) Absolute (total intake/day) or **B)** Relative (% of total Kcal) macronutrient or fiber intake does not differ between lean and obese participants. Data are represented as Mean ± S.E.M. *Represents significant difference between Lean and Obese groups at $p < 0.05$.



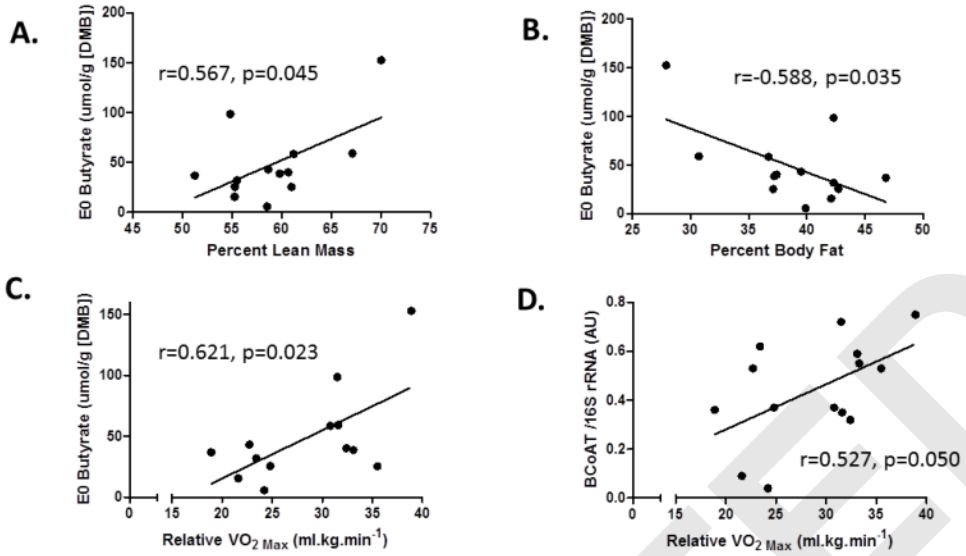
SDC 4. Rarefaction α- diversity plot. Rarefaction plot (Chao 1) at 14,201 sequences per sample (rarefaction depth). No statistical differences were observed between α-diversity of lean and obese participants at baseline or at any time point throughout the study p>0.05

Target Gene	Primer	Primer Sequence (5'-3')	Reference
BCoAT	BCoATscrF	GCIGAICATTTCACITGGAAYWSITGGCAYA TG	Louis and Flint (2007)
	BcoATscrR	CCTGCCTTTGCAATRTCICRAANGC	
mmdA	mmdAF	AATGACTCGGGIGGIGCIMGNATHCARGA	Reichardt et al. (2014)
	mmdAR	GATTGTTACYTTIGGIACNGTNGCYTC	
Universal 16S rRNA	515F	GTGYCAGCMGCCGCGGTAA	Walters et al. (2015)
	806R	GGACTACNVGGGTWTCTAAT	

SDC 3. qPCR target genes and primer sequences. Forward and reverse primers (5'-3') used gene quantification through qPCR (relative abundance vs. total 16S rRNA).



SDC 5. Random Forest classification Random Forest (RF) analysis was performed on the **A)** pre- to post-exercise change (E0-E6) and **B)** post-exercise to washout change (E6-W6) with bacterial genera as the training sets and with BMI status as the grouping variable. The cross-validation error rates (averaged across BMI groups) were compared to the baseline error rates that would be achieved by randomly assigning to either grouping variable through a Matthew's correlation coefficient.



SDC 6. SCFAs and functional genes versus baseline measures A-C) Butyrate concentrations associate with **A)** higher lean mass, **B)** lower body fat and **C)** higher cardiorespiratory fitness (VO_{2max}) within obese participants at the baseline time point (E0). **D)** Butyrate regulating gene BCoAT also associates with higher relative VO_{2max} in obese participants at E0. Pearson correlation coefficients significant at $p < 0.05$, respectively.

A.

Genus Δ E0-E6	VO_{2max} Δ E0-E6 (Spearman ρ)
Eggerthella spp.	-0.676*
f_Bifobacteriaceae spp.	-0.821**
Dorea spp.	-0.788**
Ruminococcus spp.	-0.684*
Coprobacillus spp.	-0.750**
Bifidobacterium spp.	-0.761**
Barnesiella spp.	0.833**
Lachnospira spp.	0.699*
Paraprevotella	0.723*
Veillonella spp.	0.712*

PCA factor reduction

OB group only

B.

Genus (Δ E0-E6)	$Exercise_{OBFactor1}$ loading coefficient
Eggerthella spp.	-0.674
f_Bifobacteriaceae spp.	-0.954
Dorea spp.	-0.927
Ruminococcus spp.	-0.764
Coprobacillus spp.	-0.863
Bifidobacterium spp.	-0.841
Barnesiella spp.	0.938
Lachnospira spp.	0.804
Paraprevotella	0.920
Veillonella spp.	0.905

$EXERCISE_{OB\_Factor1}$ explains 74.05 % of Variance

C.

Δ E0 –E6	$EXERCISE_{OB\_Factor1}$	Butyrate	BCoAT
% Lean Mass	0.310	0.110	0.046
% Body Fat	-0.180	-0.132	-0.46
VO_{2max}	0.843**	0.413	0.176

SDC 7. Obese microbiota changes versus $VO_{2\ max}$ A) Spearman correlations relating changes in ten bacterial genera to parallel changes in cardiorespiratory fitness within the obese group only. Spearman ρ *p<0.05, ** p<0.01 **B)** Loading coefficients for each selected taxa used in primary component factor analysis. $EXERCISE_{OB\_FACTOR1}$ is representative of the factor component that most thoroughly explained the variance in change of these select bacteria genera (74.05%). **C)** $EXERCISE_{OB\_Factor1}$ was positively associated with changes in cardiorespiratory fitness within the obese group. Pearson r *p<0.05, **p<0.01