Oligofructose-enriched inulin intake, gut microbiome characteristics and the $\dot{V}O_2$peak response to high-intensity interval training in healthy but inactive adults.

Camilla J. Williams[1], Luciana Torquati[1,2], Zhixiu Li[3], Ilaria Croci[1, 4, 5], Eliza Keating[1], Jonathan P. Little[6], Rodney A. Lea[7], Nir Eynon[8], Jeff S. Coombes[1]

[1] Centre for Research on Exercise, Physical Activity and Health, School of Human Movement and Nutrition Sciences, University of Queensland, St. Lucia, QLD, Australia

[2]Department of Sport and Health Sciences, University of Exeter, Exeter, United Kingdom

[3]Translational Genomics Group, Institute of Health and Biomedical Innovation, Brisbane, Qld, Australia

[4] Cardiac Exercise Research Group (CERG), Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[5] Department of Sport, Movement and Health, University of Basel, Basel, Switzerland

[6] School of Health and Exercise Sciences, University of British Columbia, Kelowna, BC, Canada

[7] Queensland University of Technology (QUT), Centre for Genomics and Personalised Health, Genomics Research Centre, School of Biomedical Sciences, Institute of Health and Biomedical Innovation, Qld, Australia

[8] Institute for Health and Sport (iHeS), Victoria University, Melbourne, VIC, Australia

Corresponding Author:

Jeff Coombes

School of Human Movement and Nutrition Sciences, St. Lucia, Brisbane, Queensland, 4072,

+61 7 3365 56767, jcoombes@uq.edu.au

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27 No conflict of interests.

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List of abbreviations

ASA24-Australia-2016	Automated Self-Administered 24-Hour (ASA24) Dietary Assessment Tool Australia-2016
BF%	Body fat percentage
BMI	Body mass index
BP	Blood pressure
BPM	Beats per minute
CRF	Cardiorespiratory fitness
CRP	C-reactive protein
FDR	False discovery rate
FOS	Oligofructose
GWAS	Genome Wide Association Study
HIIT	High-intensity interval training
HIIT-I	High-intensity interval training - Inulin group
HIIT-P	High-intensity interval training – Placebo group
HRmax	Maximal heart rate
Kg	Kilograms
MCID	Minimal clinically important difference
MICT	Moderate intensity continuous training
RPE	Rating of perceived exertion
SCFA	Short-chain fatty acid
Spp.	Species
$\dot{V}O_2$peak	Peak oxygen uptake
VT	Ventilatory threshold

Abstract

Background: The gut microbiome has been associated with cardiorespiratory fitness.

Objective: To assess the effects of oligofructose (FOS)-enriched inulin supplementation on the gut microbiome and the peak oxygen uptake ($\dot{V}O_2$peak) response to high-intensity interval training (HIIT).

Methods: The study was a randomized controlled trial. Forty sedentary and apparently healthy adults (n=31 females; age=31.8±9.8 years, BMI=25.9±43 $kg \cdot m^{-2}$) were randomly allocated to: i) six weeks of supervised HIIT ($3 \cdot week^{-1}$, 4x4 protocol)+12g/day of FOS-enriched inulin (HIIT-I) or ii) six weeks of supervised HIIT ($3 \cdot week^{-1}$, 38 minutes: 4x4 protocol)+12g/day of maltodextrin/placebo (HIIT-P). Each participant completed an incremental treadmill test to assess $\dot{V}O_2$peak and ventilatory thresholds (VTs), provided a stool and blood sample, and completed a 24-hour diet recall and food frequency questionnaire before and after the intervention. Gut microbiome analyses were performed using metagenomic sequencing. Fecal short-chain fatty acids (SCFA) were measured by mass spectrometry.

Results: There were no differences in the mean change in $\dot{V}O_2$peak response between groups (P=0.58). HIIT-I had a greater improvement in VTs compared to HIIT-P (VT1 - lactate accumulation: mean difference +4.3% and VT2 – lactate threshold: +4.2%, P<0.05). HIIT-I also had a greater increase in the abundance of *Bifidobacterium* taxa (False Discovery Rate (FDR) <0.05) and several metabolic processes related to exercise capacity (FDR <0.05). In both groups, secondary analysis of merged data found higher responders to HIIT (increased $\dot{V}O_2$peak ≥$3.5 mL \cdot kg^{-1} \cdot min^{-1}$) had a 2.2-fold greater mean abundance of gellan degradation pathways (FDR <0.05) and a greater but not significant abundance of *B. Uniformis* spp. (P<0.00023, FDR= 0.08).

Conclusions: FOS-enriched inulin supplementation did not potentiate HIIT-induced improvements in $\dot{V}O_2$peak, but led to gut microbiome changes possibly associated with greater ventilatory threshold improvements in healthy but inactive adults. Gellan degradation pathways and *B.uniformis* spp. were associated with greater $\dot{V}O_2$peak responses to HIIT.

Clinical Trials Register: ACTRN12618000501246.

Keywords: gut microbiome, $\dot{V}O_2$peak trainability

Background

Cardiorespiratory fitness (CRF, typically measured as peak oxygen uptake [$\dot{V}O_2$peak]) is one of the best predictors of chronic disease risk and mortality [1], and regular aerobic exercise training is recommended to improve $\dot{V}O_2$peak [2]. High intensity interval training (HIIT) is considered more time efficient and enjoyable, and elicits greater training adaptations than traditional moderate intensity continuous training [3-5]. However, there is large variability in the $\dot{V}O_2$peak response to any given exercise training, with some individuals not improving beyond random variation [6-11]. Predicting and exploring ways to induce a clinically meaningful $\dot{V}O_2$peak training response to HIIT may contribute to greater individual health outcomes.

In the HERITGAGE study [12], 85% of the variability in $\dot{V}O_2$peak response was attributed to the combined factors of genetic diversity (47%), technical error and day-to-day variability (20%), training effort (6%), age, sex, weight and ethnicity (2-3% each), and baseline $\dot{V}O_2$peak (2%) [13]. Early candidate gene studies and genome wide association studies (GWAS) [10], including our recent GWAS [14] using data (n=507) from the Predict HIIT study [15], have not found a robust panel of genetic variants associated with $\dot{V}O_2$peak response to exercise training. Thus, the use of exercise-related genes to inform clinical practice remains unsolved.

The gut microbiome is our second genome, and contains 150 times more genes than the human genome [16]. Found mainly in the colon, the gut microbiome is involved in many processes, such as digestion, production of essential vitamins, hormones, neurotransmitters and immunity [16-20]. A recent study suggests the gut microbiome is associated with aerobic capacity [21.]. The mechanism behind these associations is still unknown but might depend on gut microbiome metabolites, such as short-chain fatty acids (SCFA) [22]. SCFA,

100 including butyrate, acetate and propionate, are produced by intestinal fermentation of non-
101 digestible carbohydrates [23]. An increased production of SCFA is associated with improved
102 blood flow, improved insulin sensitivity, enhanced fatty acid and glucose metabolism, higher
103 oxidative phosphorylation, mitochondrial biogenesis and increased skeletal muscle mass [24,
104 25]. Enhancing these functions complements delivery, uptake and utilization of oxygen, and
105 therefore may increase $\dot{V}O_2$peak. Cross sectional studies have shown a higher $\dot{V}O_2$peak is
106 associated with greater abundance of butyrate producing bacteria [26],
107 *Firmicutes:Bacteroidetes* ratio [27-29], and greater microbiome diversity [26, 30, 31].
108 Intervention studies have found *Bacteroides* [32] in elderly females, and certain species in
109 adults with obesity (*Barnesiella, Lachnospira, Paraprevotella, Veillonella)* [33] to be
110 positively associated with $\dot{V}O_2$peak response following 6 and 12 weeks of continuous
111 endurance exercise training, respectively. Gut microbiome associations related to HIIT are
112 currently unknown.

113 The gut microbiome can be largely manipulated by diet [34, 35]. Soluble fermentable fibers
114 (prebiotics), such as fructo-oligosaccharide (FOS) and inulin, can change the composition
115 and activity of the gut microbiome by increasing beneficial gut bacteria and SCFA
116 production [36-38]. Inulin combined with FOS supplementation ranging from 5 -16g/day,
117 over a duration of three to nine weeks, increased *Bifidobacteria* and SCFA production in
118 healthy and clinical populations [39-41]. A diet high in fermentable fiber has also been
119 associated with greater gut microbial diversity [42], and increased butyrate producing species
120 via cross-feeding interactions [43]. In mice, a high fermentable fiber intake increased SCFA
121 acid production and exercise endurance via energy metabolism pathways [44]. Thus, a higher
122 fermentable fiber diet may improve energy production and usage, physiological functions,
123 peripheral adaptations to exercise and overall exercise capacity. Human research in this area
124 remains limited.

125 The aim of this study (Improve-HIIT) was to investigate whether the $\dot{V}O_2$peak response to six
126 weeks of high-intensity interval training could be potentiated by fermentable fiber
127 supplementation. We hypothesized that 12 g of FOS-enriched inulin daily for 6 weeks would
128 increase the availability of fermentable fibers and associated gut species resulting in greater
129 $\dot{V}O_2$peak gains.

130

Methods

Study design

This study was a randomized controlled trial, where 40 inactive (<1 hour of structured exercise each week), apparently healthy participants were randomly allocated and divided equally to one of two groups: 1) six weeks of supervised HIIT (38 minutes in total: five minutes warm up, four minutes at 90-95% heart rate maximum followed by three minutes recovery repeated another three times (i.e., 4x4 protocol), 3 x per week) + oligofructose (FOS)-enriched inulin supplementation (12 g/day); or 2) six weeks of supervised HIIT (3 x per week) + placebo (maltodextrin) supplementation (12 g/day). Participants were blinded as to which supplement they received and each participant received the same HIIT protocol at each session. All participants signed a consent form and ethical approval was obtained from the Institutional Human Research Ethics Approval committee at the University of Queensland, Australia (approval number 2018000398). The study was registered with the Australian New Zealand Clinical Trials Registry (ANZCTR) trial identification: ACTRN12618000501246.

Participants were recruited through university and clinical exercise physiology marketing channels, such as Facebook, flyers and e-newsletters. Eligibility was open to inactive male and female adults aged 18-50 years. Adults over the age of 50, as well as active adults, were excluded from the study to create a more homogeneous group for testing. Prior to baseline testing, participants completed the Adult Pre-exercise Screening System (APSS) [45]. Further exclusion criteria were based on factors that may alter the gut microbiome composition or affect participant safety. Participants were excluded if they: 1) had used antibiotics six months prior to the intervention period, 2) consumed pre or probiotic supplements within four weeks of participating in the study, 3) were pregnant, 4) had an existing cardiac condition or were at increased risk of a cardiovascular disease event due to clustering of risk factors, 5)

had recent surgery or an orthopedic condition that prevented them from exercising, 6) had diabetes, 7) had an allergy to soy, milk, egg, inulin or fructans, maltodextrin or other polysaccharides, or 8) had a chronic infection, auto-immune disease or intestinal chronic condition.

Supplementation

In the two weeks preceding the six-week HIIT intervention, each group gradually increased the dose of supplementation (fiber or placebo) from 2 g to 12 g each day (6 g each day twice daily). This was done to reduce potential side-effects associated with increasing fiber intake too quickly, such as flatulence and bloating. Participants then consumed 12 g each day (6g each day twice daily; once in the morning and once in the evening) for six weeks. Please see supplemental methods for further information regarding the supplement.

High intensity interval training (HIIT)

Following the two-week supplementation adjustment period, each group completed a 6-week HIIT exercise intervention using the 4 x 4-minute protocol [46]. Participants in both groups completed three supervised exercise sessions each week (18 sessions in total). Please see supplemental methods for further information regarding the HIIT design.

Outcome measures

All outcome measures were assessed at baseline and repeated within one week of completing the six-week HIIT intervention. Within one week was required to avoid detraining that can occur after 12 days of no exercise [47, 48]. Participants were asked to avoid making any physical activity or dietary changes during the intervention period.

Primary outcome measures

Cardiorespiratory fitness ($\dot{V}O_2$peak)

Participants completed a graded exercise treadmill test to voluntary exhaustion using the Bruce Ramp Protocol [49] with expired air analyzed using indirect calorimetry (Parvo Medica True One 2400 System, Parvo Medics, Inc., Sandy, Utah, USA). At exhaustion, the test time, respiratory exchange ratio (RER) and maximum heart rate were recorded. $\dot{V}O_2$peak was defined as the mean of the highest two 30-second epoch values [49]. The test was concluded when they reached volitional fatigue. Exercise capacity (time-on-test) was calculated as the time at which the participant stopped the test/volitional fatigue.

Gut microbiome composition and metabolic function

At baseline and following the HIIT intervention, participants were provided with two home stool collection kits. Participants were instructed to collect their stool sample the day before each $\dot{V}O_2$peak test. The first was for short-chain fatty acid analysis with instructions from the International Human Microbiome Standards for frozen samples [50]. On return, this sample was stored at -80^{O}C prior to analysis. The second kit was for metagenomic analysis.

Stool sample DNA extraction, sequencing and bioinformatic profiling

DNA was extracted on the QIAcube HT using the QIAamp 96 PowerFecal QIAcube HT Kit (Qiagen, Netherlands) [51]. For further details regarding sequencing, please see supplemental methods.

Short-chain fatty acids (SCFA) analysis

SCFA were analyzed using procedures outlined in Garcia-Villalba et al. (2012) [52]. Results were expressed as the amount of SCFA in mmol per gram of wet fecal weight. This was corrected for internal standard recovery relative to the amount of internal standard used to establish the standard curve. The amount, in µmol, of each SCFA was then expressed as a

relative percentage of the overall SCFA present (again, in μmol per gram) in each respective sample.

Secondary outcome measures (supplemental methods)

Statistical analysis

Sample size and randomization

The sample size was based on the change in relative $\dot{V}O_2$peak between the HIIT-I and HIIT-P groups. Considering participants recruited were a healthy but sedentary population, it was assumed baseline $\dot{V}O_2$peak would be 35 $mL \cdot kg^{-1} \cdot min^{-1}$. Both groups in this study were to receive HIIT, therefore it was anticipated both groups would achieve a clinically meaningful improvement in $\dot{V}O_2$peak following the training period (3.5 $mL \cdot kg\text{-}^{1}.min^{-1}$) [53]. As there are no longitudinal studies assessing gut microbiome manipulation and response to HIIT, cross-sectional study data were used for the sample size calculation assumptions [14]. In this study, those with a higher $\dot{V}O_2$peak had greater butyrate production and alpha diversity. Therefore, it was anticipated the HIIT-I group would have a 40% greater mean improvement (1.5 $mL \cdot kg^{-1}.min^{-1}$) than the HIIT-P group in $\dot{V}O_2$peak. The standard deviation (SD) of the change in both groups was assumed to be 1.5 $mL \cdot kg^{-1}.min^{-1}$. Based on these assumptions, 34 participants were required to achieve a power of 0.8, 0.05 significance (two-sided) and effect size of 1.0. Forty participants were recruited to account for a 15% loss to follow-up.

Online software [54] was used by a researcher not directly involved in the study to generate the randomization sequence using random permuted blocks with sequentially numbered opaque envelopes used to allocate participants. Over 80% adherence was required for inclusion in analysis (no more than two missed exercise sessions, heart rate / RPE meeting exercise training protocol for more than 80% of each session, no more than two missed supplement intakes and a valid $\dot{V}O_2$peak test).

$\dot{V}O_2$peak, physiological, exercise capacity, biochemical measures, nutrition intake, short chain fatty acid production

Data were tested for normality and homoscedasticity using a Shapiro-Wilk and Levene's test respectively (P<0.05). Where required, data were log-transformed. Data are presented as Mean±SD unless otherwise stated. Mean energy, macronutrient and fiber intake were calculated from the two 24-hour diet recalls and food frequency questionnaires. To test the reliability of each dietary assessment method, a two-way mixed intraclass correlation coefficient was calculated. The 24-hour diet recall mean intakes were also compared between study groups using an independent t-test. The mean fiber intake from the 24-hour diet recall was used as a covariate in analysis. Changes in body composition, physiological, exercise test, biochemical measures and fecal short chain fatty acid production between groups were compared using an analysis of covariance (ANCOVA). Covariates were selected based on factors that may influence outcome measures. Covariates included age, sex, baseline $\dot{V}O_2$peak, baseline body-fat percentage and mean fiber intake from pre and post food diaries. Medications were not added as a covariate due to the small number of participants on medications and due to the different types of medications taken. Any missing data was removed before analysis. Post-hoc testing used Tukey's least significance difference test. A P-value ≤ 0.05 was considered statistically significant.

Gut microbiome changes

Primary analysis – difference between study groups (HIIT-I and HIIT-P)

Comparisons between HIIT-I and HIIT-P study arms were calculated using a paired difference analysis (pre-treatment data points subtracted from post- treatment datapoints) on unadjusted data, and data with covariates included in analysis (age, sex, baseline $\dot{V}O_2$peak,

baseline body-fat percentage and mean fiber intake from pre and post food diaries). These covariates were based on factors that may influence the microbiome.

Exploratory analysis – pooling data

Exploratory analysis of all participants combined and stratified by $\dot{V}O_2$peak response was completed. A higher responder was defined as achieving an increase >3.5mL/kg/min and a lower responder as ≤3.5 $mL \cdot kg^{-1}.min^{-1}$. This criterion is considered clinically significant, as a one MET (3.5 $mL \cdot kg^{-1}.min^{-1}$) difference in $\dot{V}O_2$peak was associated with an 8-15% decrease in all-cause mortality over a 24-year follow-up period in over 37,112 healthy adults [53]. The first analysis looked at the difference in mean relative abundance between higher and lower responders, and the second subtracted pre-treatment from post-treatment abundance.

Analysis was completed on pooled unadjusted data, and data with covariates included in analysis (age, sex, total fiber intake (including the supplement), mean body fat percentage and baseline $\dot{V}O_2$peak). Differentially abundant microbial functions, family, phyla, genera and species between groups were identified using an ANOVA on square root transformed abundance data. Changes in fecal short chain fatty acid production were compared using an analysis of covariance (ANCOVA).

Primary and exploratory analysis

Taxonomic profiles were analyzed using supervised (i.e., guided response variable analysis for pattern discovery) multivariate methods. Adonis and Redundancy Analysis (RDA) was used to assess if variance in microbial community composition could be attributed to the study condition. Adonis was run on Bray-Curtis dissimilarities (where 1 indicates no shared species and 0 indicates all shared species).

Differential gene expression analysis (DESeq2) and ANOVA-like differential expression (Aledx2) were run on read count data. ALDEx2 used subsampling (Bayesian sampling) to

estimate the underlying technical variation. For each subsample instance, transformed data was statistically compared across study groups and computed *P* values were corrected for multiple testing using the Benjamini–Hochberg procedure.

A Fisher's exact test was used to test for differences in the presence and absence (detection rate) of microbial functions, phyla, family, genus and species across study groups. The expected *P* value (mean *P* value) was reported, which would likely have been observed if the same samples had been run multiple times (false discovery rate – FDR).

Alpha diversity for each study arm (inulin vs placebo and pooled data based on response) was measured by the Shannon index and species richness (total number of bacterial families present in each sample). Shannon index accounted for the relative abundance and evenness of the families present and quantified the entropy of microbial communities. Data was rarefied to 3234742 reads. An ANOVA of rarefied reads was used to compare the total and change in Shannon diversity and richness between study groups following the intervention period.

An FDR less than 0.05 was considered statistically significant. Statistical analysis was completed using SPSS (version 25.0, SPSS Inc., Chicago, IL, USA), and the RStudio package version 3.5.2 (RStudio, Boston, Massachusetts, USA).

Results

Figure 1 shows that from 99 interested participants, 40 (n=31 females; age=31.8±9.8) were randomized and completed the intervention. Baseline characteristics for each group are in listed **table 1**.

297 **Primary Analysis Outcomes**

298 ***Comparison of $\dot{V}O_2$peak response between HIIT-I and HIIT-P***

299 **Table 2** provides ANCOVA results for between-group tests. Both groups achieved a
300 clinically significant increase in $\dot{V}O_2$peak (> 3.5 mL·kg^{-1}.min^{-1}) following the HIIT
301 intervention, however there was no significant between-group difference (*P*=0.58). The
302 waterfall plot in **figure 2** shows that the variability in $\dot{V}O_2$peak response was similar for
303 participants in each group.

304 ***Comparison of gut microbiome composition changes between HIIT-I and HIIT-P***

305 Following the intervention, the change in abundance of several taxa and functions were
306 significantly different between groups (**supplementary table 1**). For example, the HIIT-I
307 group had a significantly greater increase (FDR <0.05) in the abundance of *Actinobacteria,*
308 *Bifidobacteriaceae* and *Bifidobacterium* taxa than the HIIT-P group. **Supplementary figure**
309 **1** shows the greater change in the abundance of the *Bifidobacterium* taxa in HIIT-I compared
310 to HIIT-P. There were several species with a large fold change in abundance between groups
311 (*P*<0.05), however, these changes were not significant following FDR-adjusted analyses
312 (FDR > 0.8). There was no significant difference in the Shannon index (0.02, 95% CI -0.2 to
313 0.4, *P*=0.68) or richness changes (4.8, 95% CI -5.1 to 14.9, *P*=0.31) between groups.

314 ***Comparison of gut microbiome metabolic function changes between HIIT-I and HIIT-P***

315 *Short-chain fatty acids*

316 The unadjusted analysis found the HIIT-P group had a 15.6% (3.4 µmol·g^{-1}) reduction in the
317 total amount of SCFAs produced, whereas HIIT-I had a 14.7% (µmol·g^{-1})) increase in total
318 SCFA production following the intervention. When adjusted for covariates, the difference
319 between groups (+14.4 µmol·g^{-1}) higher in the HIIT-I group) was not significant (*P*=0.13).

Whilst the HIIT-I group had a 4.5% greater production of acetic acid than the HIIT-P group, this too, was not significant following co-variate adjusted analysis (P=0.37). There were no other significant differences in SCFA changes between groups.

Comparison of metabolic pathways and groups changes between HIIT-I and HIIT-P

Supervised redundancy analysis found microbial functional pathways contributed to 50% of the variance between HIIT-I and HIIT-P (P=0.009). For example, the HIIT-I group had a greater increase in the change in abundance of the glucose biosynthesis and sucrose degradation pathways (P-value <0.001, FDR <0.05, Table 3). When pathways were based on the MetaCyc database, these groupings contributed to approximately 3% of the variation between HIIT-I and HIIT-P (P=0.034). **Figure 3** is a heat map detailing the clustering of functional pathways across participants. The HIIT-I group had a greater increase for the change in abundance of several pathways, such as the pentose phosphate pathway, amino acid biosynthesis, fatty acid and lipid biosynthesis, co-factor prosthetic group electron carrier and vitamin biosynthesis and carbohydrate degradation (P<0.01, FDR <0.05).

Secondary Analysis Outcomes

Comparison of exercise capacity changes between HIIT-I and HIIT-P

There were no significant between-group differences (P=0.37) in time-on-test (table 2). However, table 2 and **supplementary figure 2** outlines between-group differences for VT. Following the HIIT intervention, HIIT-I had a significantly greater increase in VT1 and VT2 (% of $\dot{V}O_2$peak) than HIIT-P (P<0.05). HIIT-I also had a significantly greater increase in $\dot{V}O_2$ at VT1 than HIIT-P (P=0.003).

Comparison of body composition, physiological and biochemical changes between HIIT-I and HIIT-P

Following the intervention, there were no significant between-group differences ($P \geq 0.05$) in body composition, physiological measures (heart rate, blood pressure) or biochemical changes, such as blood lipids, inflammatory markers and blood glucose levels (table 2).

Comparison of mean energy, macronutrient and fiber intake between dietary assessment tools and between HIIT-I and HIIT-P groups

The 24-hour diet recall and FFQ demonstrated moderate (0.7) to excellent (>0.9) intraclass correlation for total energy, macronutrient and fiber intake in both the HIIT-I and HIIT-P group (**supplementary table 2**). Based on the mean of the 24-hour diet recalls, there were no statistically significant between-group differences with energy (P=0.1), carbohydrate (P=0.2), protein (P=0.2), fat (P=0.3) or fiber intake (P=0.5).

Exploratory analysis outcomes – gut microbiome

Mean abundance comparison between higher and lower responders

Data were pooled from both groups and stratified based on $\dot{V}O_2$peak response. Supervised redundancy analysis found that collectively, genus taxa explained approximately 4.6% (P=0.03), and species 11% (P=0.003) of the variance between higher (n=21, >3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) and lower responders (n=19, ≤3.5 $mL \cdot kg^{-1} \cdot min^{-1}$) to training. For example, higher responders to training had a 9.4-fold greater mean abundance of *Bacteroides_A* genera (P<0.0001, FDR=0.12), and a 2.2-fold greater mean abundance of covariate-adjusted *Bacteroides Uniformis* spp. (P-value<0.001, FDR = 0.08) than lower responders. Higher responders to training also had a 2.2-fold greater mean abundance of the gellan degradation pathway (P<0.00001, FDR <0.05, **supplementary table 3**).

Changes following the HIIT intervention – comparison between higher and lower responders

An ANOVA of paired analysis (post-pre intervention measures) found there were no significant differences in the change in abundance of square-root transformed taxa,

membrane transport proteins, MetaCyc groups and MetaCyc pathways between higher and lower responders following the HIIT intervention (FDR adjusted *P*-value ≥0.05). Similarly, there were no significant between-group differences in Shannon index changes (-0.02, 95% CI -0.3 to 0.3, *P*=0.99) or richness changes (1.3, 95% CI -8.3 to 10.5, *P*=0.88) between higher and lower responders to training. There were also no significant differences in total SCFA (0.6 $\mu mol \cdot g^{-1}$), 95% CI-16.4 to 17.6, *P*=0.95) or proportion of individual SCFA production (% of total, *P*>0.1) between higher and lower responders to training.

Discussion

This is the first study investigating the influence of a fermentable fiber supplement on $\dot{V}O_2$peak trainability and the gut microbiome. It was found that FOS-enriched inulin supplementation did not significantly potentiate the $\dot{V}O_2$peak response to high-volume HIIT compared to a placebo, but did improve ventilatory thresholds. The response to HIIT was associated with particular microbiome characteristics, including abundance of the gellan degradation pathways and *B.uniformis* spp.

The FOS-enriched inulin group increased VT1 and VT2 by 4.3% and 4.2% (% of $\dot{V}O_2$peak) respectively, compared to the placebo group. Improvements in the VTs indicate that an individual can exercise at a higher intensity for a longer period of time before fatiguing and are strong predictors for endurance performance [55]. VT1 is where lactate starts to increase above resting levels, and occurs ~40-60% $\dot{V}O_2$peak [56]. VT2 indicates metabolic acidosis [57] and the respiratory compensation point, and presents ~70-80% $\dot{V}O_2$peak [56]. The increased VTs with the FOS-enriched inulin may be attributable to functional gut microbiome changes associated with increased availability of carbohydrates from the supplement that could be used for fermentation. The HIIT-I group had a significantly greater abundance of microbiome processes involved in energy production and usage, such as glucose biosynthesis and sucrose degradation. The HIIT-I group also had a greater increase in the change in the abundance of the pentose phosphate pathways (assists with skeletal muscle glucose metabolism [58] and counteracts oxidative stress [59]) and fatty acid and lipid biosynthesis (which can maximize fat oxidation [60]) pathways. The increase in these processes and pathways may be attributed to *Bifidobacterium* abundance changes in the HIIT-I group [61].

Specifically, the HIIT-I group had a significant increase (38-fold greater change) in the abundance of *Bifidobacterium* taxa, which coincided with a 14.4 $\mu mol \cdot g^{-1}$ greater production of total SCFA, and a 4.5% greater production of acetic acid compared to the placebo. Whilst

these SCFA improvements were not significant, the changes do complement previous research [38]. Inulin feeds *Bifidobacterial* species, which in turn increases SCFA production, and in particular acetic acid [38]. Animal models have found acetic acid can replenish glycogen in skeletal muscle during exercise [62, 63]. In mice, acetic acid improves endurance performance and this is associated with increases in the expression of genes involved in oxidative metabolism, fatty acid oxidation and muscle fiber transformation from glycolytic to oxidative fiber types [64]. Low exercise tolerance is seen in mice that cannot use acetate as a substrate for acetyl-CoA [65], and subsequently mitochondrial respiration. Therefore, it is speculated from these findings that participants in the FOS-enriched inulin group may have had a greater ability to oxidize fat at a higher workload and exercise at a higher intensity before the onset of lactate acid accumulation, resulting in the improvement in ventilatory thresholds. Future research could test these findings in an athletic population to investigate if inulin has ergogenic benefits.

Despite these findings, there were no significant effects of the FOS-enriched inulin on gut diversity or butyrate and other SCFA between groups. Based on previous research, it was expected a high fermentable fiber diet would lead to greater gut diversity [42], and that the FOS-enriched inulin would increase *Bifidobacterium* species and butyrate producing species via cross feeding interactions [38]. Similar to previous findings in mouse studies, it was anticipated a greater production of butyrate may have stimulated increased mitochondrial function and biogenesis [66, 67], and ultimately enhance changes in $\dot{V}O_2$peak following exercise training. However, our findings of a lack of an effect on $\dot{V}O_2$peak may be a result of the supplementation being a single fermentable fiber source. A recent review also found that studies using single source fermentable fibers generally failed to increase gut diversity [68]; a variety of fiber sources is better associated with overall microbiome diversity [69]. Furthermore, a recent study suggested in-vitro findings may not also transfer to in-vivo, or a longer study time (i.e. longer than six weeks) may be required for FOS-enriched inulin to

promote cross-feedings to butyrate producers [70]. A combination of supplements/fibers and the provision of probiotics (*Ruminoccus bromii* or *Clostridums chartababidum*) to feed off these fibers may also help to yield a greater butyrogenic effect [70] and more significant effects on $\dot{V}O_2$peak.

However, pooled exploratory data found there was a difference between higher and lower responders for $\dot{V}O_2$peak training response to HIIT. The higher responders had a significantly greater mean abundance of the gellan degradation pathways, which may contribute to improve energy production pathways and improved $\dot{V}O_2$peak. Gellan is a water-soluble polysaccharide found in many packaged foods, dairy products, jams, processed meats and fortified drinks [71, 72]. The final products of gellan degradation include 4-deoxy-L-threo-hex-4-enopyranuronate [71], which is further degraded to pyruvate (which can be catabolized into acetyl-CoA, lactate or succinate and ultimately metabolized into SCFA) and glyceraldehyde 3-posphate (co-factor for enzymatic reactions). Guar gum has similar properties to gellan and an early study found only *Bacteroides* species, including *B. uniformis*, were able to degrade and use the gum as an energy source [73]. With this in mind, higher responders to HIIT also had a 9.4-fold greater mean abundance of *Bacteroides_A*, and 2-fold greater mean abundance of *B.uniformis* spp. compared to lower responders. *Bacteroides A*. has previously been shown to be associated with $\dot{V}O_2$peak [32]. Furthermore, *B.uniformis* was found in greater abundance in Japanese male long-distance runners, and correlated with a greater swim time to exhaustion in mice [74]. In summary, it seems *B.uniformis* spp. may be a potential marker for health, exercise performance and $\dot{V}O_2$peak response, and warrants further investigation.

Pooled exploratory data also found there were no differences in SCFA production or gut diversity between higher or lower responders to training, which contradicts previous studies which reported a correlation with gut diversity, SCFA production and $\dot{V}O_2$peak [26, 31]. This previous research has predominantly been cross-sectional and investigated cohorts with

widely varying degrees of physical activity levels and dietary habits; these are factors that can significantly influence gut diversity [28, 75-77] and potentially SCFA production, which may have biased the results. Our cohort was more homogenous being inactive at baseline with no significant differences in macronutrient intake

Strengths and limitations

There are several limitations that need to be considered. Firstly, the cohort was predominantly female and Caucasian, and consequently, results may be biased toward this population. We did not account for menstrual cycles when completing the $\dot{V}O_2$peak tests, nor did we exclude women taking the oral contraceptive pill. There is evidence these factors may influence the observed response [78-80], but our strategy was to increase external validity in this randomized study and not to attempt to explicitly control for menstrual cycle. Secondly, we measured fecal SCFA levels only, which may not reflect production and absorption. While this provided us with an accepted estimate of gut lumen concentrations, it limited our ability to assess SCFA peripheral effects. Future studies should incorporate measurement of peripheral concentrations of SCFA in addition to fecal sampling [81]. Thirdly, fiber intake was based on food recalls, and it is well-known that self-reporting assessment tools can be inherently biased [82]. However, this assessment method is effective at estimating usual diet intake, and we found there was moderate to excellent reliability when comparing the food frequency questionnaire with the 24-hour food recall. Finally, there may have been confounding not included in analysis or measurement error that had an impact on findings [83] . Additionally, type I error (incorrectly rejecting a true hypothesis) may have been increased through multiple comparison analysis [84]. A larger sample size may have reduced some of these biases and resulted in more significant findings.

Conclusion and future directions

Although FOS-enriched inulin supplementation did not potentiate the HIIT-induced improvements in $\dot{V}O_2$peak, it did improve ventilatory thresholds. Analyzing the variability of the $\dot{V}O_2$peak response found there were specific microbiome characteristics associated with higher responders, which should be further investigated in larger studies.

Data Availability Statement

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any researcher.

Ethics Statements

Patient Consent for Publication: Obtained

Ethics Approval: Ethical approval was obtained from the Institutional Human Research Ethics Approval committee at the University of Queensland (#2018000398).

Author Contributions:

CW and JC contributed to the conception and design of the study. CW was the lead investigator and organized the database. EK was an investigator involved with the study. Microba Life Sciences completed the metagenomics analysis and bioinformatics for metagenomic data. CW completed the short-chain fatty acid analysis. CW completed remaining statistical analysis. CW wrote the first draft of the manuscript. NE provided critical comments to the manuscript writing. All authors contributed to manuscript revision, read and approved the submitted version.

Conflict of Interest

No conflict of interests.

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Supplementary data available.

Supplementary tables, figures and supplemental methods are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents available on the Journal homepage.

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718 **TABLES**

719

720 **Table 1:** Baseline participant characteristics of 40 healthy but inactive adults participating in
721 the Improve-HIIT study[1]

Characteristic	HIIT-I	HIIT-P
	n=20	n=20
Sex, *Male/Female*	5/15	4/16
Age, *years*	33.2 ± 9.8	30.4 ± 9.8
Systolic blood pressure, *mmHg*	114.8 ± 10.7	111 ± 10.5
Diastolic blood pressure, *mmHg*	70.3 ± 9.6	68.7 ± 5.5
Body Mass Index, $kg \cdot m^{-2}$	24.7 ± 3.7	27.2 ± 4.8
$\dot{V}O_2$peak, $L \cdot min^{-1}$	2.5 ± 0.6	2.2 ± 0.6
$\dot{V}O_2$peak, $ml \cdot kg \cdot min^{-1}$	35.5 ± 5.2	29.4 ± 7.3
Exercise capacity (time-on-test, minutes:seconds)	11:21 ± 1:28	11:12 ± 1:29
Medications		
Contraception	n=3	n=5
Anxiety/depression	n=2	n=4
Asthma	n=2	n=2
Blood pressure	n=2	n=1
Hormone replacement	n=0	n=2

722 [1]Values are mean ± SD unless otherwise stated, HIIT=high intensity
723 interval training, I = inulin, P = placebo, $\dot{V}O_2$peak = cardiorespiratory fitness

724

725 **Table 2:** Body composition, physiological, exercise test and biochemical measures of 40
726 healthy but inactive adults participating in the Improve-HIIT study[1].

Characteristic[3]	HIIT-I		HIIT-P		8-week change[1] mean difference between groups: HIIT-P – HIIT-I (95% CI)	*P*-value of 8-week change[2] mean difference between groups
	Baseline n=20	8 Weeks n=20	Baseline n=20	8 Weeks n=20		
Body mass, *kg*	71.2 ± 13.7	71.6 ± 13.6	75.4 ± 13.6	74.7 ± 13.6	-0.3 (-0.8 to 1.4)	0.78
BMI, $kg \cdot m^{-2}$	24.7 ± 3.7	24.6 ± 3.7	27.2 ± 4.8	26.9 ± 4.8	-0.2 (-0.3 to 0.6)	0.51
Waist, *cm*	79.7 ± 13.7	78.8 ± 10.7	80.2 ± 10.1	78.8 ± 9.1	0.9 (-0.9 to 2.7)	0.97
Hip, *cm*	100.9 ± 6.7	100.8 ± 6.4	106.2 ± 10.6	100.2 ± 23.3	-5.7 (-15.3 to 4.0)	0.22
Body Fat, *%*	36.3 ± 5.9	36.3 ± 6.3	42.4 ± 6.7	41.3 ± 6.8	-0.8 (-1.9 to 0.3)	0.17
Resting heart rate, *bpm*	66.2 ± 11.3	65.6 ± 2.4	70.8 ± 8.4	68.6 ± 11.3	0.3 (-6.5 to 5.9)	0.67
Peak heart rate, *bpm*	179.0 ± 12.0	177.8 ± 10.8	186.0 ± 9.0	184.3 ± 10.5	2.1 (-2.2 to 6.4)	0.62
Resting systolic BP, *mmHg*	114.8 ± 10.7	116.3 ± 1.9	111.0 ± 10.5	112.9 ± 9.9	-0.3 (-6.5 to 5.9)	0.28
Resting diastolic BP, *mmHg*	70.3 ± 9.6	70.9 ± 7.8	68.7 ± 5.5	71.6 ± 9.4	0.7 (-5.4 to 6.7)	0.83
$\dot{V}O_2$peak, $ml \cdot kg \cdot min^{-1}$	35.5 ± 5.2	39.2 ± 6.0	29.4 ± 7.3	33.1 ± 8.0	-0.9 (-4.7 to 2.5)	0.58
$\dot{V}O_2$peak, $L \cdot min^{-1}$	2.5 ± 0.6	2.8 ± 0.5	2.2 ± 0.6	2.5 ± 0.6	-0.03 (-0.3 to -0.3)	0.85
VT1, $ml \cdot kg \cdot min^{-1}$	16.3±2.5	21.4±4.05	14.7 ± 3.2	17.0 ± 3.4	-2.9 (-4.5 to -1.3)	0.003**
VT1, %, $\dot{V}O_2peak$	46.3 ± 5.5	54.8±6.2	50.9 ± 8.1	52.6 ± 7.1	-4.3 (-8.1% to -4.6)	0.018*
VT2, $ml \cdot kg \cdot min^{-1}$	26.9 ± 4.5	32.8 ± 6.1	22.4 ± 5.5	25.9 ± 6.0	-3.2 (-6.9 to 0.5)	0.08
VT2, % $\dot{V}O_2peak$	76.3±9.5	83.5±6.2	76.7 ± 6.6	78.8 ± 7.1	-4.2 (-0.9 to -0.03)	0.04*
Exercise capacity: time-on-test, *mm:ss*	11:21 ± 1:28	12:59 ± 1:55	9:59 ± 1:08	11:12 ± 1:29	-00:32 (-01:28 to 01:02)	0.37
Total cholesterol, $mmol \cdot L^{-1}$	4.4 ± 0.7	4.3 ± 0.7	5.1 ± 0.9	4.9 ± 0.8	0.3 (-0.1 to 0.6)	0.15
HDL cholesterol, $mmol \cdot L^{-1}$	1.4 ± 0.4	1.5 ± 0.3	1.5 ± 0.5	1.4 ± 0.4	0.1 (-0.1 to 0.3)	0.34
LDL cholesterol, $mmol \cdot L^{-1}$	2.9 ± 0.8	2.8 ± 0.7	4.1 ± 0.9	3.9 ± 1.0	0.2 (-0.4 to 0.7)	0.47
Triglycerides, $mmol \cdot L^{-1}$	1.1 ± 0.7	1.1 ± 0.7	1.1 ± 0.4	1.2 ± 0.4	0.1 (-0.1 to 0.3)	0.26
Blood glucose, $mmol \cdot L^{-1}$	5.0 ± 0.5	5.1 ± 0.5	4.9 ± 0.6	5.0 ± 0.5	0.04 (-0.7 to 0.8)	0.92
C-reactive protein, $mmol \cdot L^{-1}$	1.5 ± 1.1	1.6 ± 0.9	6.7 ± 5.3	3.6 ± 2.9	0.70 (-1.47 to 1.61)	0.65

727 [1]Adjusted for baseline measures, age, sex, mean fiber intake and body fat percentage. Values are mean ± standard deviation
728 unless otherwise stated.
729 [2]ANCOVA: * Significantly different between groups (P<0.05), **Significantly different between groups (P<0.01)
730 [3]BP = blood pressure, bpm = beats per minute, HDL= high density lipoprotein cholesterol, HIIT= high intensity interval training,
731 I=Inulin, P=placebo, kg=kilograms, mm:ss = minutes: seconds, LDL = low density lipoprotein, $\dot{V}O_2$peak = cardiorespiratory fitness,
732 VT1 = first ventilatory threshold, VT2=second ventilatory threshold.

Figure Titles

Figure 1: CONSORT flow diagram for the Improve-HIIT study

Figure 2: Waterfall plot showing the $\dot{V}O_2$peak response of each participant in the Improve-HIIT study

Figure 3: Top differentiated (P<0.05) functional pathways in 40 healthy but inactive adults based on study group (HIIT-P and HIIT-I) and response to training.

Abundances were scaled to maximum read of 1. High = higher responders to HIIT (>3.5 mL·kg-¹.min-¹), Low = lower responders to HIIT (≤3.5 mL·kg-¹.min-¹), HIIT=high intensity interval training, I=inulin, P=placebo

Supplementary Data

Supplemental Tables

Supplemental table 1: Top-most differentially abundant changes (pre to post intervention) in functions and taxa between groups of 40 healthy but inactive adults participating in the Improve-HIIT study[1]

Function/Taxa	Mean HIIT-I (read counts) n=20	Mean HIIT-P (read counts) n=20	Between group difference in fold change[3]	Unadjusted *P*-value	Unadjusted FDR[4,5] value	Covariate[2]-adjusted *P*-value	Covariate[2]-adjusted FDR[4,5] value
Phyla							
Actinobacteria	5.4 ± 6	-0.34 ± 2.6	0.063	0.00044	0.0044**	0.00025	0.0025**
Family							
Bifidobacteriaceae	5.3 ± 5.5	0.14 ± 2.1	38	0.00056	0.03*	0.00027	0.014*
Genus							
Bifidobacterium	5.3 ± 5.5	0.14 ± 2.1	38	0.00056	0.09	0.00027	0.046*
Anaerostipes	1.3 ± 1.3	0.1 ± 0.53	13	0.0055	0.47	0.0049	0.42
CAG-74 MIC3751	-0.0082 ± 0.037	0.023 ± 0.044	2.8	0.025	0.89	0.0012	0.68
CAG-352	-0.079 ± 0.28	0.088 ± 0.28	1.1	0.049	0.89	0.036	0.87
Species							
s__Anaerostipes hadrus	1.1 ± 1.3	0.08 ± 0.51	14	0.0056	0.84	0.0048	0.84
s__Bifidobacterium longum	1 ± 1.4	0.097 ± 0.57	10	0.018	0.84	0.012	0.84
s__CAG-74 MIC3751	-0.0082 ± 0.037	0.023 ± 0.044	2.8	0.025	0.84	0.012	0.84
s__Coprococcus eutactus	0.0049 ± 0.016	-0.048 ± 0.089	9.8	0.027	0.84	0.024	0.84
s__Butyricicoccus MIC5408	-0.019 ± 0.063	-0.069 ± 0.093	-3.6	0.028	0.84	0.032	0.84
Membrane transport proteins							
The Bile Acid:Na⁺ Symporter (BASS) Family (2.A.28.2.6)	4. $x10^{-6} \pm 6.9x10^{-6}$	$1.2x10^{-7} \pm 2.1x10^{-6}$	40	0.0015	0.31	0.00056	0.20
The Voltage-gated Ion Channel (VIC)	$4.8x10^{-6} \pm 6.6x10^{-6}$	$1x10^{-7} \pm 1.7x10^{-6}$	48	0.0011	0.31	0.00076	0.20

Superfamily (1.A.1.5.25)							
The Uncharacterized 9 or 10 TMS Protein (U-TMP) Family (9B.226.1.8)	$6.3x10^{-6} \pm 7.7x10^{-6}$	$5.9x10^{-7} \pm 3.0x10^{-6}$	11	0.0015	0.31	0.0011	0.20
The Tight Adherence (Pilus) Biogenesis Apparatus (TABA) Family (9.A.47.2.2)	$2x10^{-5} \pm 2.7x10^{-5}$	$1.6x10^{-6} \pm 8.4x10^{-6}$	13	0.0025	0.31	0.0013	0.20
The ATP-binding Cassette (ABC) Superfamily (3.A.1.135.6)	$1.1x10^{-5} \pm 1.4x10^{-5}$	$1.9\ x10^{-7} \pm 5.7x10^{-6}$	58	0.0035	0.31	0.0018	0.20
Functional Groups/Pathways							
Pentose Phosphate Pathways	$0.00013 \pm 9.5x10^{-5}$	$-6.6\ x10^{-5} \pm 0.0002$	0.51	0.00099	0.031*	0.00095	0.018*
C1 Compound Utilization and Assimilation	$0.00026 \pm 3\ x10^{-4}$	-0.00017 ± 0.00046	0.65	0.0013	0.031*	0.00097	0.018*
Amino Acid Biosynthesis	0.0013 ± 0.0017	-0.00037 ± 0.0016	0.28	0.0025	0.032*	0.0011	0.018*
Cofactor Prosthetic Group Electron Carrier and Vitamin Biosynthesis	0.00096 ± 0.00094	-0.00051 ± 0.0019	0.53	0.0038	0.036*	0.0024	0.029*
Fatty Acid and Lipid Biosynthesis	0.00095 ± 0.00094	-0.00031 ± 0.0015	0.33	0.0027	0.032*	0.0035	0.034*
Carbohydrate Degradation	0.00072 ± 0.00071	-0.00031 ± 0.0014	0.43	0.0046	0.037*	0.0061	0.049*
MetaCyc Pathways							
PWY-7343~UDPα-D-glucose biosynthesis I	$5.7x10^{-5} \pm 5.6x10^{-5}$	$-2.1x10^{-5} \pm 5.2x10^{-5}$	0.37	$7.2\ x10^{-5}$	0.033*	$7.4x10^{-5}$	0.045*
PWY-3801~sucrose degradation II (sucrose synthase)	$4.7x10^{-5} \pm 4.6x10^{-5}$	$-1.3x10^{-6} \pm 3.9x10^{-5}$	0.23	0.00012	0.033*	0.00011	0.045*
PWY-5384~sucrose degradation IV (sucrose phosphorylase)	$5.5x10^{-5} \pm 5.4x10^{-5}$	$-6x10^{-6} \pm 3.5x10^{-5}$	0.11	0.00012	0.050	0.00017	0.047*
PWY-5156~superpathway of	$4.9x10^{-5} \pm 6.1x10^{-5}$	$-2.1x10^{-5} \pm 6x10^{-5}$	0.43	0.00049	0.05	0.001	0.05

fatty acid biosynthesis II (plant)							
NONOXIPENT-PWY~phosphate pathway (non-oxidative branch)	$3.6x10^{-5} \pm 3.1x10^{-5}$	$-4.3x10^{6} \pm 3.8x10^{-5}$	0.12	0.0012	0.06	0.001	0.05
PENTOSE-P-PWY-pentose phosphate pathway	$3.8x10^{-5} \pm 2.9x10^{-5}$	$-2.0\ x10^{-5} \pm 6.2\ x10^{-5}$	0.53	0.001	0.05	0.00097	0.05

[1]Values are mean ± SD unless otherwise stated.
[2]Covariates include baseline $\dot{V}O_2$peak, baseline body fat, sex, age, mean fiber intake (g/day)
[3]Fold change = difference in abundance change between study groups
[4] Paired difference analysis (pre-treatment data points subtracted from post- treatment datapoints): *Significantly different between groups FDR (P<0.05), **Significantly different between groups FDR (P<0.01)
[5]FDR = false discovery rate, HIIT = high intensity interval training, HIIT-I=inulin group, HIIT-P=placebo group.

Supplemental table 2. Mean intake of macronutrients and fibre intake across dietary assessment tools and between groups of 40 healthy but inactive adults participating in the Improve-HIIT study[1]

Macronutrient	HIIT – P[4] n=20				HIIT – I[4] n=20				Difference between study groups[3]	*P*-value
	Mean of baseline and 8-weeks		Intraclass correlation[2]	F-test significance	Mean of baseline and 8-weeks		Intraclass correlation[2]	F-test significance		
	FFQ	24-hour diet recall			FFQ	24-hour diet recall				
Total energy intake, *kJ·day⁻¹*	6976.0 ± 1481.0	7195.2 ± 1769.9	0.90 (0.62 to 0.97)	0.001	7635.7 ± 1442.2	8151.2 ± 1813.2	0.78 (0.21 to 0.94)	0.01	-956.0 (-2112.3 to 200.3)	0.1
Fibre intake, *g·day⁻¹*	20.3 ± 8.1	20.7 ± 6.8	0.89 (0.62 to 0.97)	0.001	23.5 ± 6.1	22.1 ± 5.1	0.70 (0.14 to 0.88)	0.01	-1.4 (-5.2 to 2.5)	0.5
Carbohydrate, *g·day⁻¹*	175.6 ± 57.6	172.7 ± 40.9	0.82 (0.27 to 0.96)	0.01	181.6 ± 47.8	190.3 ± 43.4	0.77 (0.21 to 0.94)	0.03	-17.6 (-44.6 to 9.4)	0.2
Protein, *g·day⁻¹*	68.3 ± 11.1	73.1 ± 16.1	0.80 (0.17 to 0.96)	0.02	63.8 ± 19.8	64.9 ± 22.1	0.78 (0.1 to 0.94)	0.02	8.2 (-4.2 to 20.6)	0.2
Fat, *g·day⁻¹*	93.9 ± 30.7	82.9 ± 30.7	0.80 (0.29 to 0.94	0.009	87.0 ± 33.2	93.2 ± 34.3	0.84 (0.56 to 0.95)	0.0005	-10.3 (-31.1 to 10.5)	0.3

[1] Values are mean ± standard deviation unless otherwise stated
[2] Intraclass correlation coefficient between FFQ and 24-hour diet recall assessment tools: Intraclass correlation (95% CI)
[3] T-test of 24-diet recall intake means: HIIT-P – HIIT-I (95% CI).
[4] HIIT = high intensity interval training, I = inulin, P = placebo

Supplemental table 3: Pooled exploratory data analysis of the mean abundance (pre and post intervention data points) of taxa between higher (>3.5 $mL \cdot kg^{-1}.min^{-1}$) and lower responders (≤ 3.5 $mL \cdot kg^{-1}.min^{-1}$) to HIIT in 40 healthy but inactive adults participating in the Improve-HIIT study[1]

Function	Mean Low Responders (read counts) n=20	Mean High Responders (read counts) n=20	Between group difference in fold change[3]	Unadjusted *P*-value	Unadjusted FDR[4,5] value	Covariate[2]-adjusted *P*-value	Covariate[2]-adjusted FDR[4,5] value
Family							
Bacteroidaceae	1.05 ± 7.9	20.0 ± 8	-1.3	0.0037	0.2	0.0025	0.13
CAG-727	0.15 ± 0.41	0.02 ± 0.089	7.5	0.025	0.51	0.021	0.44
Anaerovoracaceae	0.053 ± 0.086	0.019 ± 0.049	2.8	0.029	0.51	0.026	0.44
Genus							
Bacteroides_A	0.18 ± 0.47	1.7 ± 2.8	-9.4	0.0007	0.11	0.0007	0.12
Parasutterella	0.1 ± 0.28	0.35± 0.45	-3.5	0.0028	0.24	0.004	0.26
Erysipelatoclostridium	0 ± 0	0.039 ± 0.09	-Inf	0.0043	0.24	0.0046	0.26
CAG-1427	0.026 ± 0.058	0.084 ± 0.13	-3.2	0.024	0.46	0.019	0.34
Megamonas	0.15 ± 0.4	0 ± 0	Inf	0.024	0.46	0.013	0.34
Species							
s__Bacteroides uniformis	1.6 ± 2.1	3.5 ± 2.9	-2.2	0.0004	0.0002	0.1	0.08
s__Parasutterella excrementihominis	0.05 ± 0.14	0.32 ± 0.42	-6.4	0.0006	0.0009	0.1	0.15
s__Bacteroides_A plebeius_A	0 ± 0	1.1 ± 2.3	-Inf	0.0017	0.0021	0.19	0.23
s__Collinsella aerofaciens	0.5 ± 0.73	0.14 ± 0.35	3.6	0.0076	0.0066	0.49	0.4
s__ER4 MIC3169	0.047 ± 0.11	0 ± 0	Inf	0.0084	0.49	0.0044	0.36
s__Faecalibacterium prausnitzii_A	2 ± 2.1	1.1 ± 1.7	1.8	0.012	0.4	0.013	0.49
Membrane Transport Proteins							
The Glycan-binding Protein (SusD) Family 8.A.46.11	$1.8x10^{-6} \pm 2.7x10^{-6}$	$6x10^{-6} \pm 7.3x10^{-6}$	-3.3	4.8×10^{-5}	0.032*	5.6×10^{-5}	0.04*
The Outer Membrane Receptor (OMR) Family 1.B.14.6.13	$1.9x10^{-6} \pm 2.5x10^{-6}$	$6x10^{-6} \pm 7.2x10^{-6}$	-3.3	9.0×10^{-5}	0.032*	0.0001	0.041*

The Outer Membrane Receptor (OMR) Family1.B.14.6.1	$3.5 \times 10^{-6} \pm 4.8 \times 10^{-6}$	$9.7 \times 10^{-6} \pm 1.2 \times 10^{-5}$	-2.8	0.0002	0.037*	0.0003	0.041*
The Glycan-binding Protein (SusD) Family 8.A.46.2.2	$1.5 \times 10^{-6} \pm 2.1 \times 10^{-6}$	$5.7 \times 10^{-6} \pm 9.9 \times 10^{-6}$	-3.8	0.0002	0.037*	0.0003	0.041*
The Outer Membrane Receptor (OMR) Family 1.B.14.14.1	$2.6 \times 10^{-6} \pm 4 \times 10^{-6}$	$7.6 \times 10^{-6} \pm 9.6 \times 10^{-6}$	-2.9	0.0003	0.037*	0.0003	0.041*
Groups							
TCA cycle	0.0014 ± 0.00031	0.0013 ± 0.00034	-1.1	0.018	0.27	0.021	0.27
Polymeric Compound Degradation	0.0016 ± 0.00045	0.0013 ± 0.00045	-1.2	0.022	0.27	0.022	0.27
Carboxylate Degradation	0.00083 ± 0.00035	0.00069 ± 0.00031	-1.2	0.03	0.27	0.033	0.27
Inorganic Nutrient Metabolism	0.0037 ± 0.00054	0.0034 ± 0.00071	-1.1	0.032	0.27	0.029	0.27
Acetyl-CoA Biosynthesis	$0.00025 \pm 8.8 \times 10^{-5}$	$0.00021 \pm 8.4 \times 10^{-5}$	-1.2	0.04	0.27	0.038	0.27
Pathways							
PWY-6827~gellan degradation	$5.1 \times 10^{-5} \pm 5.8 \times 10^{-5}$	$0.00011 \pm 6.8 \times 10^{-5}$	-2.2	4.4×10^{-5}	0.036*	3.1×10^{-5}	0.025*
ASPARAGINE-DEG1-PWY-1~L-asparagine degradation III (mammalian)	$0.00015 \pm 9.4 \times 10^{-5}$	$0.00021 \pm 5.5 \times 10^{-5}$	-1.4	0.0006	0.24	0.0003	0.09
PWY-7625~phosphatidylinositol biosynthesis II (eukaryotes)	$2.6 \times 10^{-5} \pm 1.6 \times 10^{-5}$	$3.8e\text{-}05 \pm 1.6 \times 10^{-5}$	-1.5	0.0009	0.24	0.0003	0.09
PWY-6970~acetyl-CoA biosynthesis II (NADP-dependent pyruvate dehydrogenase)	$7.8e \times 10^{-5} \pm 6.5 \times 10^{-5}$	$0.00012 \pm 6.4 \times 10^{-5}$	-1.5	0.0032	0.42	0.003	0.42
PWY-6594~superpathway of Clostridium acetobutylicum solventogenic fermentation	$1.5 \times 10^{-5} \pm 5.1 \times 10^{-5}$	$7.2 \times 10^{-5} \pm 0.00011$	-4.8	0.0032	0.42	0.0029	0.42

[1]Values are mean ± SD unless otherwise stated.
[2]Covariates include baseline $\dot{V}O_2$peak, baseline body fat, sex, age, mean fiber intake (g/day)
[3]Fold change difference in abundance between lower and higher responders across all samples (pooled exploratory data).
[4]ANOVA: *Significantly different between higher and lower responders FDR (P<0.05), **Significantly different between higher and lower responders FDR (P<0.01)
[5]FDR = false discovery rate, HIIT = high intensity interval training

Supplementary Figure Titles

Supplemental figure 1: Differences between groups in the change of Bifidobacterium abundance in 40 healthy but inactive adults following the HIIT-P and HIIT-I interventions (adjusted for age, sex, baseline $\dot{V}O_2$peak, mean fibre ($g \cdot day^{-1}$) and mean body fat percentage). *Differences based on paired difference analysis, significantly different change between groups (FDR <0.05). HIIT=high intensity interval training, I=inulin, P=placebo.

Supplemental figure 2: Change in ventilatory threshold (VT) response (% of $\dot{V}O_2$peak) in 40 healthy but inactive adults following the HIIT-P and HIIT-I interventions.
The ends of each box represent the upper and lower quartiles; the median is marked by the horizontal line within each box; the mean is represented by the cross, the whiskers represent the lower and upper extremes; the dots represent individual data points.
*Differences based on ANCOVA: significantly different change between groups (P<0.05), adjusted for age, sex, baseline $\dot{V}O_2$peak, baseline body fat percentage and mean fibre intake ($g \cdot day^{-1}$). HIIT=high intensity interval training, I=inulin, P=placebo

Supplemental Methods

Prebiotic Supplement

A prebiotic fiber (Prebiotin, Jackson GI Medical Institute America, Harrisburg, Pennsylvania, USA) [1] was selected as it has been identified as a high quality supplement and is being used in three National Institute of Health studies [2-4]. Prebiotin is a FOS-enriched inulin, which is a combination of longer and shorter chain inulin derived from chicory root. The recommended intake is 2 g each day, progressing to 12 g or more per day as tolerated. This recommendation provided by Prebiotin is consistent with previous studies that have shown increased bifidogenic bacteria and SCFA production with a combined inulin and FOS supplementation ranging from 5-16 g each day, over a duration of three to nine weeks [5-7]. Each supplement was in a powder form and could be taken with any liquid (hot or cold). Supplements were packaged in a clear, non-labelled jar. Each jar contained a 4 g spoon. Compliance, bowel symptoms and tolerance were monitored through a diary.

HIIT design

A HIIT design for six weeks was chosen to optimize $\dot{V}O_2$peak changes within a relatively short time-frame [8]. Heart rate (Polar FT1, Kempele, Finland) and rating of perceived exertion (RPE) were monitored throughout the session. Participants were instructed to be within their target heart range (90-95% of peak heart rate identified from the $\dot{V}O_2$peak test), which correlated with an RPE of ‘hard’ to ‘very hard’, by the second minute of each 4-minute high-intensity interval period. To increase heart rate or RPE, the intensity was increased either through speed, incline or a mixture of the two. Adherence to the protocol was monitored via a supervising accredited exercise physiologist and recorded on a hard copy document.

Stool sample DNA extraction, sequencing and bioinformatic profiling

Sequencing was performed to a target depth of 3Gbp (2Gbp minimum, approximately 7-16 M paired-end reads) raw read generation before quality filtering. Data quality was guaranteed at 75% and above reads >Q30 at the completion of the sequencing run. Human DNA reads were first removed by aligning all reads with Burrow-Wheeler Aligner [9] to the Human Genome assembly GRCh38.p12. The taxonomic profile was generated by using the Microba Community Profiler v2.0.2 (MCP: [10][85]) and the Microba Genome Database v2.0.0 (MGDB). MGDB adopts the taxonomic descriptions from the Genome Taxonomy Database (GTDB) [11][86]. Genomospecies within MGDB that are not present in GTDB are assigned a species identifier suffixed with a MIC (species assigned by Microba, Brisbane, Australia). Such species are unique to MGDB (for example CAG-74 MIC3751) and are composed of uncultured organisms. Relative abundances were calculated by MCP and represent the fraction of microbial cells of each species in the community.

Ventilatory threshold

Ventilatory threshold values were assessed following completion of the $\dot{V}O_2$peak test and taken from the metabolic cart. These were compared with manual calculations from two assessors using the v-slope method [12]. The median of the three assessments was then used. The first ventilatory threshold (VT1), an indication of initial significant lactate accumulation, was measured at the first change in slope of VCO_2/VO_2. The second ventilatory threshold (VT2), indicating a lactate threshold, was measured at the second change in slope of VCO_2/VO_2 [13].

Blood analysis

Participants were instructed to refrain from caffeine, tobacco and exercise in the 24 hours prior to exercise testing, as well as to fast overnight and be well-hydrated in preparation for the fasted blood test. Analysis of total, HDL and LDL cholesterol, triglycerides, glucose and

C-reactive protein (CRP) from plasma or serum were measured on an automated clinical chemistry analyzer (Randox Datomer Plus, Randox Laboratories, Crumlin, Antrim, UK) using the manufacturer's procedures.

Body composition

Body fat percentage was completed following the fasted blood test and measured using dual-energy x-ray absorptiometry (DEXA, Hologic QDR Series, Massachusetts, USA). Body mass, height, waist and hip circumferences were measured using standard procedures [14].

Following the fasted blood collection and DEXA scan, and one hour before exercise testing, participants were given a standardized meal consisting of either a 250 mL Up & Go milk-based nutritional drink (Sanitarian, Berkeley Vale, NSW, Australia), and/or a 270 g fruit muesli bar (Carmen's Fine Foods, Cheltenham, Victoria, Australia) based on participant preference. Energy intake was replicated at post testing. Both these snacks have a similar nutritional composition. The Up & Go and the Carmen's Muesli bar had 815 kJ and 768 kJ of energy, 28.7 g and 25.1 g of carbohydrate, 4.2 g and 6.7 g of fat, and 8.2 g and 4.1 g of protein per serve, respectively.

Nutritional data

After the collection of stool samples, participants were instructed by a nutritionist to complete a validated 24-hour dietary recall [15]. for The ASA24-Australia-2016 analysis provides a total of nutrients and food groups and lists the mean consumption of macro and micronutrients. The ASA24-Australia-2016 was completed on a computer by participants at baseline and following the intervention period, immediately after stool collection and before each $\dot{V}O_2$peak test. A nutritionist assisted the participant where needed. The dietary recall included supplements taken outside of the intervention. For the metagenomic analysis, participants were required to complete an Australian-specific but yet to be validated food frequency questionnaire (developed by Microba, Brisbane, Australia), day of sampling

questionnaire (e.g., time of sample collection, stress status, sleep status, medication use in the lead up to sample), medical history and mental health questionnaire immediately following the collection of each stool sample. These were used by the company completing the analysis (Microba, Brisbane, Australia) to provide a detailed report to each participant.

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