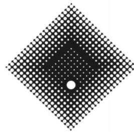

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Oligofructose-enriched inulin intake, gut microbiome characteristics and the $\dot{V}O_2$peak response to high-intensity interval training in healthy inactive adults.

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26 No conflict of interests.

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39

40 **List of abbreviations**

Abbreviation	Definition
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

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42

43

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±4.3 kg·m^{-2}) were randomly allocated to: i) six weeks of supervised HIIT (4x4 min bouts at 85–95% HRpeak, interspersed with 3 min of active recovery, 3·week^{-1}) + 12 g·day^{-1} of FOS-enriched inulin (HIIT-I) or ii) six weeks of supervised HIIT (3·week^{-1}, 4x4 min bouts) + 12 g·day^{-1} 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 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 than HIIT-P (VT1 - lactate accumulation: mean difference +4.3% and VT2 – lactate threshold: +4.2%, *P*<0.05). HIIT-I 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). Exploratory analysis of merged data found participants with a greater response to HIIT ($\dot{V}O_2$peak ≥3.5mL·kg^{-1}·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).

68 **Conclusions:** FOS-enriched inulin supplementation did not potentiate HIIT-induced

69 improvements in $\dot{V}O_2$peak, but led to gut microbiome changes possibly associated with

70 greater ventilatory threshold improvements in healthy inactive adults. Gellan degradation

71 pathways and *B.uniformis* spp. were associated with greater $\dot{V}O_2$peak responses to HIIT.

72 **Clinical Trials Register:** ACTRN12618000501246.

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

74

75 **Background**

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

85 In the HERITGAGE study (12), 85% of the variability in $\dot{V}O_2$peak response was attributed to
86 the combined factors of genetic diversity (47%), technical error and day-to-day variability
87 (20%), training effort (6%), age, sex, weight and ethnicity (2-3% each), and baseline
88 $\dot{V}O_2$peak (2%) (13). Early candidate gene studies and genome wide association studies
89 (GWAS) (10), including our recent GWAS (14) using data (n=507) from the Predict HIIT
90 study (15), have not found a robust panel of genetic variants associated with $\dot{V}O_2$peak
91 response to exercise training. Thus, the use of exercise-related genes to inform clinical
92 practice remains unsolved.

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

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

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

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

129

130 # Methods

131 **Study design**

132 This study was a randomized controlled trial, where 40 inactive (<1 hour of structured
133 exercise each week), apparently healthy participants were randomly allocated to one of two
134 groups: 1) six weeks of supervised HIIT (38 minutes in total: 4x4 min bouts at 85–95%
135 HRpeak, interspersed with 3 min of active recovery, $3 \cdot week^{-1}$) + oligofructose (FOS)-
136 enriched inulin supplementation ($12\ g \cdot day^{-1}$); or 2) six weeks of supervised HIIT (3 x per
137 week) + placebo (maltodextrin) supplementation ($12\ g \cdot day^{-1}$). Participants were blinded as to
138 which supplement they received and each participant received the same HIIT protocol at each
139 session. All participants signed a consent form and ethical approval was obtained from the
140 Institutional Human Research Ethics Approval committee at the University of Queensland,
141 Australia (approval number 2018000398). The study was registered with the Australian New
142 Zealand Clinical Trials Registry (ANZCTR) trial identification: ACTRN12618000501246.
143 Participants were recruited through university and clinical exercise physiology marketing
144 channels, such as Facebook, flyers and e-newsletters. Eligibility was open to inactive male
145 and female adults aged 18-50 years. Adults over the age of 50, as well as active adults, were
146 excluded from the study to create a more homogeneous group for testing. Prior to baseline
147 testing, participants completed the Adult Pre-exercise Screening System (APSS) (45).
148 Exclusion criteria were based on factors that may alter the gut microbiome composition or
149 affect participant safety. Participants were excluded if they: 1) had used antibiotics six
150 months prior to the intervention period, 2) consumed pre or probiotic supplements within four
151 weeks of participating in the study, 3) were pregnant, 4) had an existing cardiac condition or
152 were at increased risk of a cardiovascular disease event due to clustering of risk factors, 5)
153 had recent surgery or an orthopedic condition that prevented them from exercising, 6) had
154 diabetes, 7) had an allergy to soy, milk, egg, inulin or fructans, maltodextrin or other

155 polysaccharides, or 8) had a chronic infection, auto-immune disease or intestinal chronic
156 condition.

157 ***Supplementation***

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

164 ***High intensity interval training (HIIT)***

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

169 **Outcome measures**

170 All outcome measures were assessed at baseline and repeated within one week of completing
171 the six-week HIIT intervention. Within one week was required to avoid detraining effects
172 (47, 48). Participants were asked to avoid making any physical activity or dietary changes
173 during the intervention period.

174

175 **Primary outcome measures**

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

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

184 ***Gut microbiome composition and metabolic function***

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

190 ***Stool sample DNA extraction, sequencing and bioinformatic profiling***

191 DNA was extracted on the QIAcube HT using the QIAamp 96 PowerFecal QIAcube HT Kit
192 (Qiagen, Netherlands) (51). For further details regarding sequencing, please see
193 supplementary methods.

194 ***Short-chain fatty acids (SCFA) analysis***

195 SCFA were analyzed using procedures outlined in Garcia-Villalba et al. (2012) (52). Results
196 were expressed as the amount of SCFA in mmol·gram^{-1} of wet fecal weight. This was
197 corrected for internal standard recovery relative to the amount of internal standard used to
198 establish the standard curve. The amount, in µmol, of each SCFA was then expressed as a

199 relative percentage of the overall SCFA present (again, in µmol·gram^{-1}) in each respective
200 sample.

201 **Secondary outcome measures (supplementary methods)**

202 **Statistical analysis**

203 *Sample size and randomization*

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

218 Online software (54) was used by a researcher not directly involved in the study to generate
219 the randomization sequence using random permuted blocks with sequentially numbered
220 opaque envelopes used to allocate participants. Over 80% adherence was required for
221 inclusion in analysis (no more than two missed exercise sessions, heart rate / RPE meeting

222 exercise training protocol for more than 80% of each session, no more than two missed
223 supplement intakes and a valid $\dot{V}O_2$peak test).

224

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

227 Data were tested for normality and homoscedasticity using a Shapiro-Wilk and Levene's test
228 respectively (P<0.05). Where required, data were log-transformed. Data are presented as
229 Mean±SD unless otherwise stated. Baseline and 8-week within-group comparisons for the
230 24-hour diet recall and FFQ were analysed using a paired t-test. The mean energy,
231 macronutrient and fiber intake were calculated from the two 24-hour diet recalls (baseline
232 and 8 weeks). To test the reliability of this dietary assessment method, a two-way mixed
233 intraclass correlation coefficient was determined between the mean 24-hour diet recall data
234 and the mean FFQ data. The 24-hour diet recall mean intakes were also compared between
235 study groups using an independent t-test. Because the 24-hour recall was a validated study,
236 the mean fiber intake from the 24-hour diet recall was used as a covariate in analysis instead
237 of the mean intake identified from the FFQ. Changes in body composition, physiological,
238 exercise test, biochemical measures and fecal short chain fatty acid production between
239 groups were compared using an analysis of covariance (ANCOVA). Covariates were selected
240 based on factors that may influence outcome measures. Covariates included age, sex, baseline
241 $\dot{V}O_2$peak, baseline body-fat percentage and mean fiber intake from pre and post food diaries.
242 Medications were not added as a covariate due to the small number of participants on
243 medications and due to the different types of medications taken. Post-hoc testing used
244 Tukey's least significance difference test. A P-value < 0.025 was considered statistically
245 significant.

246

247 ***Gut microbiome changes***

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

249 Comparisons between HIIT-I and HIIT-P study arms were calculated using a paired
250 difference analysis (pre-treatment data points subtracted from post- treatment data points) on
251 unadjusted data, and data with covariates included in analysis (age, sex, baseline $\dot{V}O_2$peak,
252 baseline body-fat percentage and mean fiber intake from pre and post food diaries). These
253 covariates were based on factors that may influence the microbiome.

254 *Exploratory analysis – pooling data*

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

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

269 *Primary and exploratory analysis*

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

275 Differential gene expression analysis (DESeq2) and ANOVA-like differential expression
276 (Aledx2) were run on read count data. ALDEx2 used subsampling (Bayesian sampling) to
277 estimate the underlying technical variation. For each subsample instance, transformed data
278 was statistically compared across study groups and computed *P* values were corrected for
279 multiple testing using the Benjamini–Hochberg procedure.

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

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

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

293 *Missing Data*

294 There were zero missing samples for the gut microbiome, $\dot{V}O_2$peak, physiological, exercise
295 capacity, nutrition intake or short chain fatty acid production data. However, three people
296 were unable to provide a blood sample, and were excluded from the blood profile analysis.

297

298 Results

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

302 **Primary Analysis Outcomes**

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

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

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

310 Following the intervention, the change in abundance of several taxa and functions were
311 significantly different between groups (**supplementary table 1**). For example, the HIIT-I
312 group had a significantly greater increase (FDR <0.05) in the abundance of *Actinobacteria,*
313 *Bifidobacteriaceae* and *Bifidobacterium* taxa than the HIIT-P group. **Supplementary figure**
314 **1** shows the greater change in the abundance of the *Bifidobacterium* taxa in HIIT-I compared
315 to HIIT-P. There were several species with a large fold change in abundance between groups
316 (P<0.05), however, these changes were not significant following FDR-adjusted analyses

317 (FDR > 0.8). There was no significant difference in the Shannon index (0.02, 95% CI -0.2 to
318 0.4, *P*=0.68) or richness changes (4.8, 95% CI -5.1 to 14.9, *P*=0.31) between groups.

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

320 *Short-chain fatty acids*

321 The unadjusted analysis found the HIIT-P group had a 15.6% (3.4 $\mu mol \cdot g^{-1}$) reduction in the
322 total amount of SCFAs produced, whereas HIIT-I had a 14.7% ($\mu mol \cdot g^{-1}$)) increase in total
323 SCFA production following the intervention. When adjusted for covariates, the difference
324 between groups (+14.4 $\mu mol \cdot g^{-1}$ higher in the HIIT-I group) was not significant (*P*=0.13).
325 Whilst the HIIT-I group had a 4.5% greater production of acetic acid than the HIIT-P group.
326 This too, was not significant following covariate adjusted analysis (*P*=0.37). There were no
327 other significant differences in SCFA changes between groups.

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

329 Supervised redundancy analysis found microbial functional pathways contributed to 50% of
330 the variance between HIIT-I and HIIT-P (*P*=0.009). For example, the HIIT-I group had a
331 greater increase in the change in abundance of the glucose biosynthesis and sucrose
332 degradation pathways (*P*-value <0.001, FDR <0.05, supplementary table 1). When pathways
333 were based on the MetaCyc database, these groupings contributed to approximately 3% of the
334 variation between HIIT-I and HIIT-P (*P*=0.034). **Figure 3** is a heat map detailing the
335 clustering of functional pathways across participants. The HIIT-I group had a greater increase
336 for the change in abundance of several pathways, such as the pentose phosphate pathway,
337 amino acid biosynthesis, fatty acid and lipid biosynthesis, co-factor prosthetic group electron
338 carrier and vitamin biosynthesis and carbohydrate degradation (*P*<0.01, FDR <0.05).

339

340 **Secondary Analysis Outcomes**

341 *Comparison of exercise capacity changes and ventilatory thresholds (VTs) between HIIT-I*
342 *and HIIT-P*

343 There were no significant between-group differences (P=0.37) in time-on-test (table 2).
344 However, table 2 and **supplementary figure 2** outlines between-group differences for VTs.
345 Following the HIIT intervention, HIIT-I had a greater increase in VT1 and VT2 (% of
346 $\dot{V}O_2$peak) than HIIT-P (P<0.05). HIIT-I also had a significantly greater increase in $\dot{V}O_2$ at
347 VT1 than HIIT-P (P=0.003).

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

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

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

355 There were no significant within-group differences (P>0.08) in baseline or 8-week 24-hour
356 diet recalls for fiber or macronutrient intake in the HIIT-I or HIIT-P group **(supplementary**
357 **table 2).** Mean fiber intake from the 24-hour diet recall was used as a covariate in analysis.
358 Based on the mean of the 24-hour diet recalls, there were no statistically significant between-
359 group differences (P=0.8) with fiber intake between the HIIT-I (20.5±5.6 $g \cdot day^{-1}$) or HIIT-P
360 group (21.5±8.5 $g \cdot day^{-1}$). There were no other significant differences between the HIIT-I and
361 HIIT-P group in total energy (P=0.2), carbohydrate (P=0.5), protein (P=0.8), fat (P=0.5) or
362 fiber intake (P=0.8) using the validated 24-hour diet recall **(supplementary table 3).** The 24-
363 hour diet recall and FFQ demonstrated moderate (0.7) to good (>0.8) intraclass correlations

364 for mean total energy, macronutrient and fiber intake in both the HIIT-I and HIIT-P group
365 (supplementary table 3).

366 **Exploratory analysis outcomes – gut microbiome**

367 *Mean abundance comparison between higher and lower responders*

368 Data were pooled from both groups and stratified based on $\dot{V}O_2$peak response. Supervised
369 redundancy analysis found that collectively, genus taxa explained approximately 4.6%
370 (*P*=0.03), and species 11% (*P*=0.003) of the variance between higher (n=21, >3.5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$)
371 and lower responders (n=19, ≤3.5 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) to training. For example, higher
372 responders to training had a 9.4-fold greater mean abundance of *Bacteroides_A* genera
373 (*P*<0.0001, FDR=0.12), and a 2.2-fold greater mean abundance of covariate-adjusted
374 *Bacteroides Uniformis* spp. (*P*-value<0.001, FDR = 0.08) than lower responders. Higher
375 responders to training also had a 2.2-fold greater mean abundance of the gellan degradation
376 pathway (*P*<0.00001, FDR <0.05, **supplementary table 4**).

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

378 An ANOVA of paired analysis (post-pre intervention measures) found there were no
379 significant differences in the change in abundance of square-root transformed taxa,
380 membrane transport proteins, MetaCyc groups and MetaCyc pathways between higher and
381 lower responders following the HIIT intervention (FDR adjusted *P*-value ≥0.05).
382 Similarly, there were no significant between-group differences in Shannon index changes
383 (-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)
384 between higher and lower responders to training. There were also no significant differences
385 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
386 production (% of total, *P*>0.1) between higher and lower responders to training.

387 ## Discussion

388 This is the first study investigating the influence of a fermentable fiber supplement on
389 $\dot{V}O_2$peak trainability and the gut microbiome. It was found that FOS-enriched inulin
390 supplementation did not significantly potentiate the $\dot{V}O_2$peak response to high-volume HIIT
391 compared to a placebo, but did improve ventilatory thresholds. The response to HIIT was
392 associated with particular microbiome characteristics, including abundance of the gellan
393 degradation pathways, *Bacteroides_A* genera and *B.uniformis* spp.

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

409 Specifically, the HIIT-I group had a significant increase (38-fold greater change) in the
410 abundance of *Bifidobacterium* taxa, which coincided with a with an increase in sucrose
411 degradation pathways I and IV. These are involved in the *Bifidobacterium* shuttle, which

412 results in acetate production (62). This may explain the resulting 14.4 μmol·g^{-1} greater
413 production of total fecal SCFA, and a 4.5% greater production of acetic acid compared to the
414 placebo. Whilst these SCFA improvements were not significant, the changes do complement
415 previous research (38). Inulin feeds *Bifidobacterial* species, which in turn increases SCFA
416 production, and in particular acetic acid (38). Animal models have found acetic acid can
417 replenish glycogen in skeletal muscle during exercise (63, 64). In mice, acetic acid improves
418 endurance performance and this is associated with increases in the expression of genes
419 involved in oxidative metabolism, fatty acid oxidation and muscle fiber transformation from
420 glycolytic to oxidative fiber types (65). Low exercise tolerance is seen in mice that cannot use
421 acetate as a substrate for acetyl-CoA (66), and subsequently mitochondrial respiration.
422 Therefore, it is speculated from these findings that participants in the FOS-enriched inulin
423 group may have had a greater ability to oxidize fat at a higher workload and exercise at a
424 higher intensity before the onset of lactate acid accumulation, resulting in the improvement in
425 ventilatory thresholds. Future research could test these findings in an athletic population to
426 investigate if inulin has ergogenic benefits.

427 Despite these findings, there were no significant effects of the FOS-enriched inulin on gut
428 diversity or butyrate and other SCFA between groups. Based on previous research, it was
429 expected a high fermentable fiber diet would lead to greater gut diversity (42), and that the
430 FOS-enriched inulin would increase *Bifidobacterium* species and butyrate producing species
431 via cross feeding interactions (38). Similar to previous findings in mouse studies, it was
432 anticipated a greater production of butyrate may have stimulated increased mitochondrial
433 function and biogenesis (67, 68), and ultimately enhance changes in $\dot{V}O_2$peak following
434 exercise training. However, our findings of a lack of an effect on $\dot{V}O_2$peak may be a result of
435 the supplement being a single fermentable fiber source. A recent review also found that
436 studies using single source fermentable fibers generally failed to increase gut diversity (69); a

variety of fiber sources is better associated with overall microbiome diversity (70). 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 (71). 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 (71) and more significant effects on $\dot{V}O_2$peak.

Pooled exploratory data found there was a difference between higher and lower responders for the $\dot{V}O_2$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 (72, 73). The final products of gellan degradation include 4-deoxy-L-threo-hex-4-enopyranuronate (72), 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 (74). With this in mind, higher responders to HIIT also had a 9.4-fold greater mean abundance of *Bacteroides_A* genera, 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 (75). 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.

462 Pooled exploratory data also found there were no differences in SCFA production or gut
463 diversity between higher or lower responders to training, which contradicts previous studies
464 which reported a correlation with gut diversity, SCFA production and $\dot{V}O_2$peak (26, 31). This
465 previous research has predominantly been cross-sectional and investigated cohorts with
466 widely varying degrees of physical activity levels and dietary habits; these are factors that can
467 significantly influence gut diversity (28, 76-78) and potentially SCFA production, which may
468 have biased the results. Our cohort was more homogenous being inactive at baseline with no
469 significant differences in macronutrient intake

470 **Strengths and limitations**

471 There are several limitations that need to be considered. Firstly, the cohort was
472 predominantly female and Caucasian, and consequently, results may be biased toward this
473 population. We did not account for menstrual cycles when completing the $\dot{V}O_2$peak tests, nor
474 did we exclude women taking the oral contraceptive pill. There is evidence these factors may
475 influence the observed response (79-81), but our strategy was to increase external validity in
476 this randomized study and not to attempt to explicitly control for menstrual cycle. Secondly,
477 we measured fecal SCFA levels only, which may not reflect production and absorption.
478 While this provided us with an accepted estimate of gut lumen concentrations, it limited our
479 ability to assess SCFA peripheral effects. Future studies should incorporate measurement of
480 peripheral concentrations of SCFA in addition to fecal sampling (82). Thirdly, fiber intake
481 was based on food recalls, and it is well-known that self-reporting assessment tools can be
482 inherently biased (83). The use of self-reported measures (i.e., fiber intake) when analyzing
483 results may have caused residual confounding. However, food recalls are an effective
484 assessment method at estimating usual diet intake, and we found there was moderate to very
485 good reliability when comparing the 24-hour food recall with the FFQ. The FFQ was
486 unvalidated; however, it was only used as an estimation of usual diet, whilst the validated 24-

487 hour recall mean was used in the statistical analysis. These factors combined may hopefully
488 limit some of the bias associated with self-reporting. Additionally, there may have been other
489 confounding factors not included in analysis or measurement error that had an impact on
490 findings (84) . Finally, type I error may have been increased through multiple comparison
491 analyses with our secondary outcomes (85). As always, a larger sample size may have
492 reduced some of these biases.

493 **Conclusion and future directions**

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

498

499 **Data Availability Statement**

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

502 **Ethics Statements**

503 *Patient Consent for Publication*: Obtained

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

506 **Author Contributions:**

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

514 **Conflict of Interest**

515 No conflict of interests.

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520 **Supplementary material available.**

521 Supplementary tables, figures and supplemental methods are available from the
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760 **TABLES**

761

762 **Table 1:** Baseline participant characteristics of 40 healthy inactive adults participating in the

763 Improve-HIIT study[1]

Characteristic	HIIT-I[2]	HIIT-P[2]
	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

764 [1]Values are mean ± SD unless otherwise stated, HIIT=high intensity
765 interval training, I = inulin, P = placebo, $\dot{V}O_2$peak = cardiorespiratory fitness
766 [2]HIIT-I = 12 g inulin each day + 6 weeks of HIIT, HIIT-P=12 g placebo each day + 6 weeks of HIIT

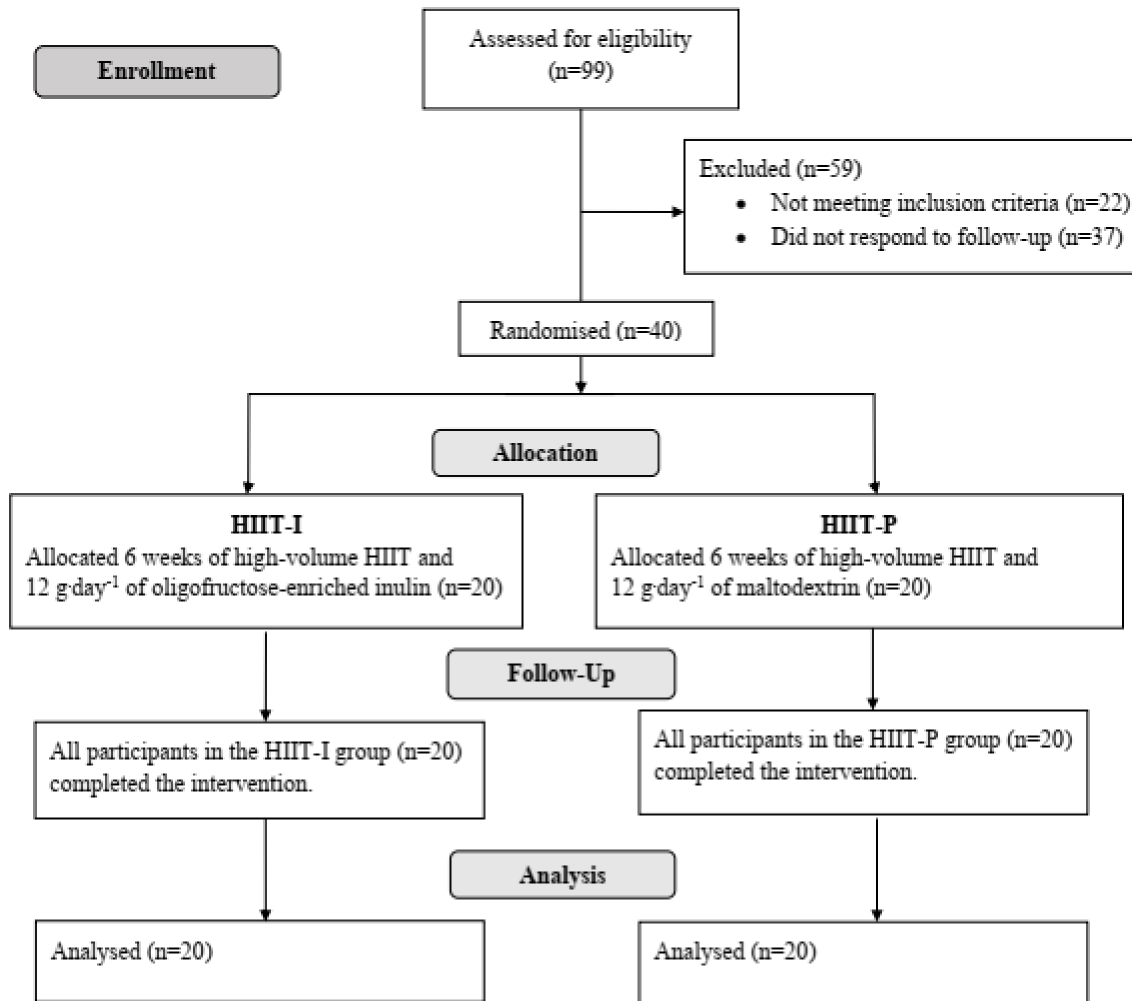
767

768 **Table 2:** Body composition, physiological, exercise test and biochemical measures of 40
769 healthy inactive adults participating in the Improve-HIIT study.

Characteristic[3]	HIIT-I[4]		HIIT-P[4]		Delta changes after 8 weeks[1] (post minus baseline values): HIIT-P – HIIT-I (95% CI)	*P*-value of 8-week change[2] in the 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

770 [1]Adjusted for baseline measures, age, sex, mean fiber intake and body fat percentage. Values are mean ± standard deviation unless otherwise
771 stated.
772 [2]ANCOVA:* Significantly different between groups (*P*<0.05), **Significantly different between groups (*P*<0.025)
773 ***Significantly different between groups (*P*<0.01)
774 [3]BP = blood pressure, bpm = beats per minute, HDL= high density lipoprotein cholesterol, HIIT= high intensity interval training, I=inulin,
775 P=placebo, kg=kilograms, mm:ss = minutes: seconds, LDL = low density lipoprotein, $\dot{V}O_2$peak = cardiorespiratory fitness, VT1 = first
776 ventilatory threshold (lactate accumulation), VT2=second ventilatory threshold (lactate threshold).
777 [4]HIIT-I = 12 g inulin each day + 6 weeks of HIIT, HIIT-P=12 g placebo each day + 6 weeks of HIIT

778 **Figure Titles**



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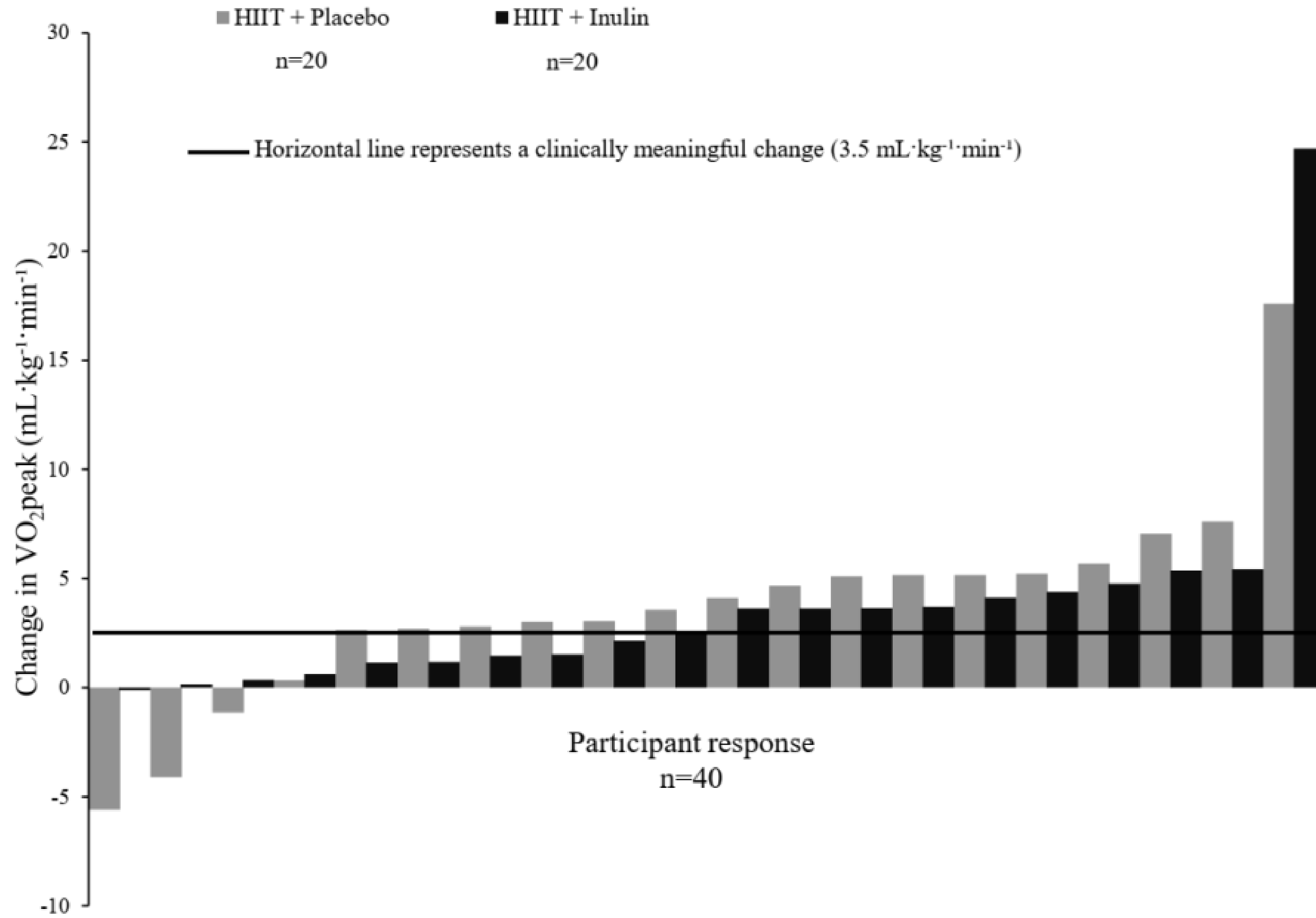
780 **Figure 1:** CONSORT flow diagram for the Improve-HIIT study



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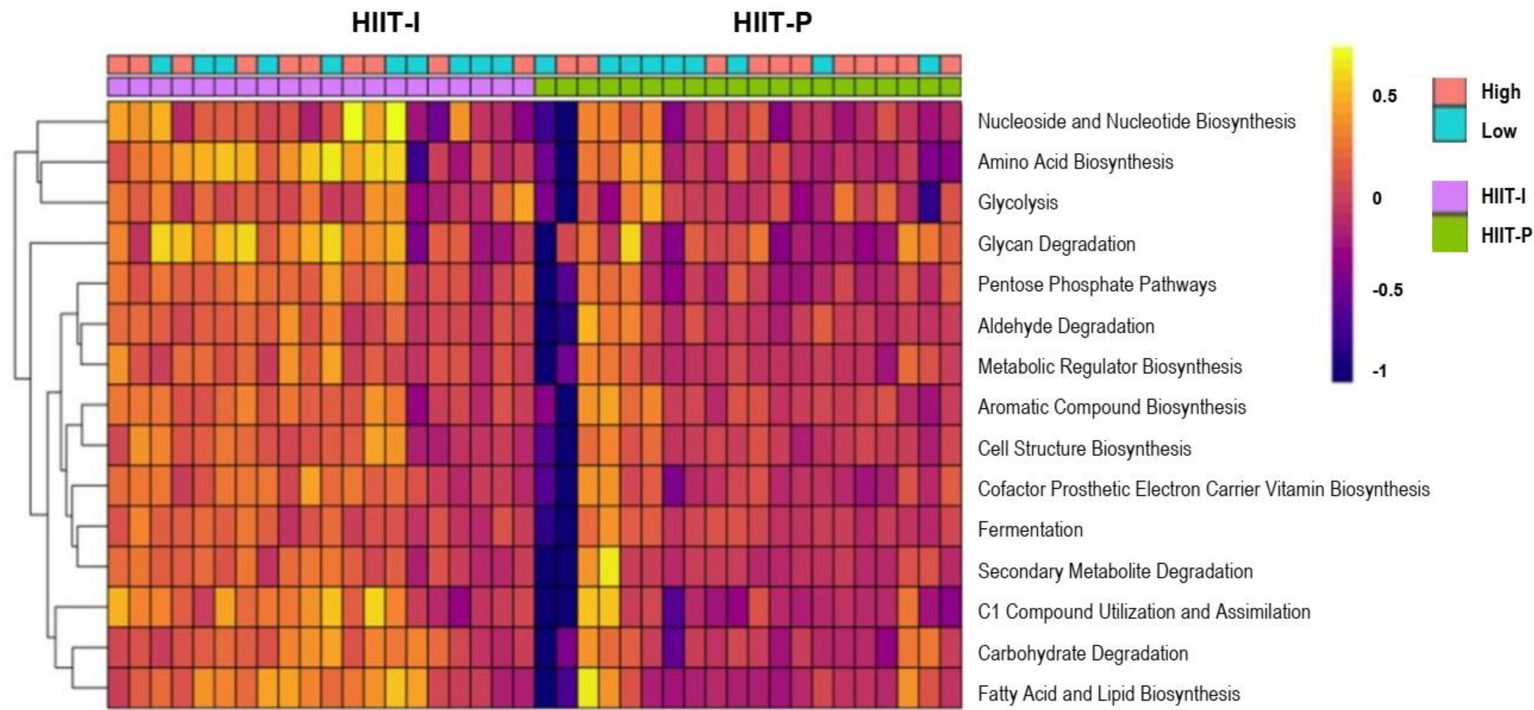
782 **Figure 2**: Waterfall plot showing the $\dot{V}O_2$peak response of each participant in the Improve-HIIT study



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

785 training.

786 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

787 mL·kg^{-1}·min^{-1}), HIIT=high intensity interval training, I=inulin, P=placebo

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