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1 **Impact of concurrent training on body composition and gut microbiota in**
2 **postmenopausal women with overweight or obesity**

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37

38 **ABSTRACT**

39 **Purpose:** Menopause tends to be associated with an increased risk of obesity and abdominal
40 fat mass (FM) and is associated with lower intestinal species diversity. The aim of this study
41 was to determine the effects of a high-intensity interval training and resistance training (HIIT
42 + RT) program on body composition and intestinal microbiota composition in overweight or
43 obese postmenopausal women.

44 **Methods:** Participants ($n = 17$) were randomized in two groups: HIIT + RT group (3 × / week,
45 12 weeks) and control group without any training. Dual-energy X-ray absorptiometry was used
46 to measure whole-body and abdominal/visceral FM and fat-free mass. Intestinal microbiota
47 composition was determined by 16S rRNA gene sequencing at baseline and at the study end,
48 and the diet controlled.

49 **Results:** Compared with sedentary controls, physical fitness (Maximal Oxygen Consumption,
50 Peak Power Output) increased, total abdominal and visceral FM decreased, and segmental

51 muscle mass increased in the training group. Although the HIIT + RT protocol did not modify
52 α -diversity and taxonomy, it significantly influenced microbiota composition. Moreover,
53 various intestinal microbiota members were correlated with HIIT + RT-induced body
54 composition changes, and baseline microbiota composition predicted the response to the HIIT
55 + RT program.

56 **Conclusions:** HIIT + RT is an effective modality to reduce abdominal/visceral FM and
57 improve physical capacity in non-dieting overweight or obese postmenopausal women.
58 Training modified intestinal microbiota composition and the response to training seems to
59 depend on the initial microbiota profile. More studies are needed to determine whether
60 microbiota composition could predict the individual training response.

61

62 **Key words:** menopause, concurrent training, fat mass, abdominal/visceral fat mass, gut
63 microbiota composition.

64

65

66 **INTRODUCTION**

67 Obesity has reached epidemic proportions and is a major contributor to the global burden of
68 chronic disease and disability. Obesity is a complex multifactorial disease mainly favored by
69 sedentary lifestyle, low physical activity level, and high consumption of processed and high-
70 calorie foods (1). Fat mass (FM) accumulation and metabolic disturbances are associated with
71 greater risk of cardiovascular diseases (CVD). It is recognized that abdominal and particularly
72 visceral adiposity increase the risk of developing obesity-related complications compared with
73 subcutaneous adiposity (2). The reduction of visceral adipose tissue is naturally associated with
74 a decreased risk of metabolic syndrome and CVD (3).

75 The prevalence of obesity is higher in post- than in pre-menopausal women (4). Menopause is
76 associated with energy metabolism changes, particularly decreased fat oxidation at rest and
77 during exercise (5), lower spontaneous physical activity (6) and consequently, lower total
78 energy expenditure (5). Estrogen production deficiency also favors muscle mass loss and FM
79 gain with higher central body fat distribution (*i.e.* abdominal/visceral deposits) and
80 consequently higher CVD risk (7, 8).

81 The combination of endurance (aerobic) and resistance (strength) exercise modalities can
82 contribute to preventing and treating obesity-associated abnormalities (9). Recent studies by
83 our laboratory showed that 3-4-month programs of high-intensity interval training (HIIT) (10)
84 or HIIT plus resistance training (RT) (11) are safe and efficient strategies to significantly
85 reduce total and (intra-)abdominal FM in women with overweight or obesity. Compared with
86 moderate intensity continuous training, the HIIT + RT combination also increases muscle mass
87 in this population (11), leading to higher resting metabolic rate and 24-h energy expenditure.
88 Recently, Rashiti et al. confirmed that compared with moderate intensity continuous training +
89 RT, HIIT + RT has a greater effect on obesity-related parameters in postmenopausal women
90 (12).

91 Gut microbial dysbiosis/unfavorable composition might also promote obesity development. It
92 is acknowledged that gut microorganisms are important pathogenic factors, contributing to
93 systemic inflammation, insulin-resistance and directly or indirectly, to body composition
94 changes (13, 14). Similarly, obesity has been associated with significant changes in gut
95 microbiota composition and metabolic functions (including a decrease of α and β diversity
96 and/or an imbalance between benefic and pathogenic bacteria that could alter the carbohydrate
97 metabolism), indicating a two-way relationship between intestinal microbiota and human
98 metabolism (14). The gut microbiota is mainly shaped by diet (13), but regular physical activity
99 is also emerging as an important modulator of gut microbial community structure and diversity

100 (15–17). Higher levels of physical activity (total volume and intensity) are associated with
101 higher fecal bacterial diversity and with the increased representation of some phyla and certain
102 short-chain fatty acids in the feces of healthy adults (18, 19). Menopause and more generally
103 aging reduce intestinal species diversity, an effect that might contribute to the high prevalence
104 of obesity in this population (20). Few studies in humans have directly examined the effect of
105 regular physical activity on gut microbiota (20–24) and none used the HIIT + RT combination.
106 Therefore, the main objective of this study was to evaluate the impact of a 12-week HIIT + RT
107 program on body composition and gut microbial community structure and diversity in non-
108 dieting postmenopausal women with overweight or obesity. We hypothesized that the
109 concurrent training HIIT + RT would decrease total and (intra-)abdominal adipose tissue and
110 that these alterations would be associated with a favorable alteration of the gut microbiota
111 composition.

112

113 **METHODS**

114 On the basis of previous results on visceral FM loss after a 3-month HIIT + RT program in
115 postmenopausal women (11), sample size was estimated before the study beginning to ensure
116 a statistical power of 80%. Considering a two-sided type I error at 5%, a minimal difference of
117 1.5 kg in visceral FM loss (standard deviation = 1.0) could be detected with $n = 7$ participants
118 per group. The sample size was increased to $n = 14$ and 15 per group to take into account
119 participants lost to follow-up.

120 This study was approved by the relevant ethics committee (Comité de Protection des Personnes
121 Ouest VI, CPP 1141 HPS1) and was registered on ClinicalTrials.gov (ClinicalTrials.Gov:
122 NCT03940924). Participants were recruited *via* flyers, posters and adverts on websites and
123 social networks. Before the study, participants were given explanations on the study aims and
124 methods and their written informed consent was collected.

125

126

127

128

129 **Participants**

130 For practical and feasibility reasons the protocol was carried out in four waves (February to
131 May and September to December 2019, February to May and September to December 2020),
132 and the third wave was stopped due to the COVID-19 pandemic.

133 Thirty-three participants were recruited according to the following inclusion criteria:
134 postmenopausal women, body mass index (BMI) $>25 \text{ kg.m}^{-2}$ and $\leq 40 \text{ kg.m}^{-2}$, and stable eating
135 habits and physical activity for at least 3 months. Non-inclusion criteria were: medical
136 contraindications to intense physical activity, painful joints, taking hormone replacement
137 therapy and taking antibiotics for at least 3 months. Finally, 29 women were selected for the
138 12-week study (Figure 1). None of them had history of chronic arterial or respiratory diseases,
139 CVD, or endocrine disorders. All participants reported low levels of physical activity, based
140 on the Global Physical Activity Questionnaire (GPAQ) result (25). Participants were
141 subdivided in two groups in order of inclusion: intervention group (HIIT + RT, $n = 14$) and
142 control group without training program (CONT, $n = 15$).

143

144 **EXPERIMENTAL DESIGN**

145

146 **Anthropometric and body composition measurements.** Body weight was measured to the
147 nearest 0.1 kg on a Seca 709 scale (Balance Seca 709, France), with participants wearing only
148 underwear. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body
149 mass index (BMI, kg.m^{-2}) was calculated as body mass (kg) divided by the square of height

150 (m²). Waist circumference (WC, cm) was measured midway between the last rib and upper
151 iliac crest, and hip circumference at the level of the femoral trochanters. Both measures were
152 taken in standing position with a measuring tape. The sagittal abdominal diameter (supine
153 abdominal height) was measured with a Holtain–Kahn abdominal caliper (Holtain Limited,
154 Crymych, Pembrokeshire, UK) to the nearest mm in the sagittal plane at the level of the iliac crests
155 (L4–L5) during normal expiration, with the subject lying supine on a firm bench with knees
156 bent. Abdominal skinfold thickness was measured at four different sites (at 12 cm and 7 cm to
157 the right and left of the navel) with a Harpenden Skinfold Caliper (Mediflex Corp., Long Island,
158 NY, USA), and the mean subcutaneous abdominal skinfold thickness was then calculated. The
159 same experienced investigator took all anthropometric measurements at baseline and after the
160 study end.

161

162 **Fat and muscle mass localization.** Total body and regional fat mass (FM) and free fat mass
163 (FFM) (expressed as kg and % of body mass) were measured with a dual-energy X-ray
164 absorptiometry scanner (QDR-4500A, Hologic, Inc., Marlborough, MA, USA). Muscle mass
165 was calculated by removing the bone mineral content from the FFM. Participants in underwear
166 were placed in the middle of the table in supine position. Whole body scanning was performed
167 for 3 min and the same operator analyzed all collected data. Two regions of interest were
168 manually isolated by the same investigator: the area from L2–L3 to the pubic rami to determine
169 the total abdominal FM (kg), and the area delineated by the upper border formed by an oblique
170 line passing through the femoral neck to the horizontal line passing through the knee to
171 determine the thigh muscle mass (kg) (26). Total visceral FM (kg) was estimated from the total
172 abdominal FM obtained by dual-energy X-ray absorptiometry, mean subcutaneous abdominal
173 skinfold thickness and abdominal height, as previously described (11).

174

175 **Preliminary visit - maximal exercise testing.** VO_{2max} was measured during a graded
176 exhaustive exercise test on a cycle ergometer (Ergoline, Bitz, Germany). After a 3-min warm-
177 up at 30 W, power output was increased by 10 Watts per minute until the participant's
178 exhaustion (the test lasted between 10 and 15 minutes after warm-up). Participants were
179 strongly encouraged by the experimenters throughout the test to perform a maximal effort.
180 Respiratory gases (VO_2 and VCO_2) were measured breath-by-breath through a mask connected
181 to O_2 and CO_2 analyzers (MasterScreenTM CPX, Care Fusion, Le Pont-de-Claix, France).
182 VO_{2max} was determined as the highest oxygen uptake during a 15s period. Ventilatory
183 parameters were averaged every 30s. Heart activity was monitored by ECG throughout the test,
184 and heart rate recorded continuously. VO_{2max} achievement was based on the following criteria:
185 1) oxygen uptake reaching a plateau with increasing work rate; 2) respiratory exchange ratio
186 values higher than 1.1; and 3) maximal heart rate (HR_{max}) within 10% of the age-predicted
187 maximal values. The Peak Power Output (PPO), expressed in watts or $watts \cdot kg^{-1}$, was
188 considered the highest power measured at VO_{2max} .

189

190

191

192 **Microbiota composition analysis by Illumina sequencing**

193 Participants received a plastic tube to collect their stool, within 24h before the study initiation
194 (first exercise session for the training group) and at the study end (24h after last exercise session
195 for the training group), and were instructed to store the stool sample in a plastic bag in their
196 home freezer. Upon receipt, samples were stabilized in RNA Later (Sigma Aldrich, MI, USA)
197 and stored at $-80^{\circ}C$ until processing.

198 Genomic DNA from fecal samples was extracted using the Maxwell[®] RSC PureFood GMO
199 and Authentication Kit (Promega, Madison, WI, USA). The 16S rRNA gene was amplified and

200 sequenced using the Illumina MiSeq technology and the Earth Microbiome Project protocol
201 with some slight modifications. Briefly, region V4 of the 16S rRNA gene was PCR-amplified
202 from each sample using composite forward and reverse primers designed with the Golay error-
203 correcting code, and used to tag the PCR products (27). The sequence of the forward primer
204 (515F) was: 5'-
205 *AATGATACGGCGACCACCGAGATCTACACGCTXXXXXXXXXXXXTATGGTAATT*
206 *GTGTGYCAGCMGCCGCGGTAA*-3'. The italicized sequence is the 5' Illumina adapter, the
207 12 X sequence is the Golay barcode, the bold sequence is the primer pad, the italicized and
208 bold sequence is the primer linker, and the underlined sequence is the conserved bacterial
209 primer 515F. The sequence of the reverse primer (806R) was: 5'-
210 *CAAGCAGAAGACGGCATACGAGATAGTCAGCCAGCCGGACTACNVGGGTWTCTA*
211 *AT*-3'. The italicized sequence is the 3' reverse complement sequence of the Illumina adapter,
212 the bold sequence is the primer pad, the italicized and bold sequence is the primer linker, and
213 the underlined sequence is the conserved bacterial primer 806R. PCR reactions included the
214 Hot Master PCR mix (Quantabio, Beverly, MA, USA), 0.2 mM of each primer, and 10–100 ng
215 template. The reaction conditions were 3 min at 95 °C, followed by 30 cycles of 45 s at 95 °C,
216 60 s at 50 °C, and 90 s at 72 °C on a BioRad thermocycler. PCR products were quantified with
217 the Quant-iT PicoGreen dsDNA assay. Then, a master DNA pool was generated from the
218 purified products in equimolar ratios and purified with Ampure magnetic purification beads
219 (Agencourt, Brea, CA, USA). The pooled product was quantified using the Quant-iT
220 PicoGreen dsDNA assay and then sequenced using an Illumina MiSeq sequencer (paired-end
221 reads, 2 × 250 bp) at the Genom'IC sequencing facility of Cornell University.
222 Then, the 16S rRNA sequences were analyzed using Quantitative Insights Into Microbial
223 Ecology (QIIME2, Flagstaff, AZCA, USA) version 2019.7. Sequences were demultiplexed and
224 quality-filtered using the Dada2 method (28) with QIIME2 default parameters to detect and

225 correct Illumina amplicon sequence data, and a table of QIIME2 artifacts was generated. Then,
226 the α -diversity of bacterial communities was assessed by calculating the Shannon's diversity
227 index, and β -diversity was used to analyze the dissimilarity among the group membership and
228 structure. Both weighted and unweighted UniFrac distances were reported according to the
229 principal coordinates analysis (PCoA). Group differences in α - and β -diversity indices were
230 calculated using the Kruskal–Wallis test and permutational multivariate analysis of variance
231 (PERMANOVA), respectively. For taxonomic analysis, features were assigned to operational
232 taxonomic units with a 99% threshold of pairwise identity to the Greengenes reference database
233 13.8 (29). Differential taxon abundance among groups was tested with the ANCOM approach
234 and the QIIME2 software. The W-value generated by ANCOM is a count of the number of
235 sub-hypotheses (Aitchison's log-ratio) that are significantly different across the tested groups
236 for a given taxon. Correlations between gut microbiota composition (initial relative abundance
237 of families) and clinical parameters were calculated with the Spearman's correlation test and
238 the GraphPad Prism software (version 7.0). Finally, the baseline fecal microbial profiles,
239 comprising 977 features, were used to build a random forest model to investigate whether the
240 intestinal microbiota profile can predict response to exercise. The receiving operating
241 characteristic (ROC) curve profile and the area under the ROC curve (AUC) were used as the
242 main indicators of the model performance.

243

244 **Biochemical assays.** Blood samples were taken one week before the program start (baseline
245 values) and then 2–4 days after the last exercise session for the trained group or after the last
246 week of the study period for the control group. After overnight fasting, a cannula was inserted
247 in the antecubital vein, and whole blood was collected in EDTA- and fluoride-containing
248 vacutainers tubes. The plasma concentration of total cholesterol, high-density lipoprotein
249 cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG),

250 ultrasensitive reactive C protein (usCRP), glucose, insulin, and glycated hemoglobin (HbA1c)
251 were immediately measured at an analysis center. The HOMA-IR index was calculated using
252 the formula: $HOMA-IR = [Fasting\ glucose\ (mmol.L^{-1}) \times Fasting\ insulin\ (\mu U.mL^{-1})] / 22.5$ (30).

253

254 **Physical activity and dietary assessments.** All participants were requested not to modify their
255 dietary and physical activity habits during the 12-week study period Their usual weekly level
256 of physical activity was determined at baseline and after the 12-week study period using the
257 French version of the GPAQ (25).

258 At baseline and at week 12, each participant filled in a 5-day food-intake diary (3 weekdays
259 and 2 weekend days) that was evaluated by a dietician using a nutrition analysis software
260 (Nutrilog[®], Marans, France). A telephone helpline was proposed to participants who
261 experienced problems in completing the 5-day food-intake diary.

262

263 **Training program.**

264 The training program included HIIT + RT three times per week, for 12 weeks (total = 36
265 sessions). Supervised sessions (approximately 45 minutes/each) were carried out at the Center
266 of Resources, Expertise and Performance in Sports (CREPS), generally on Monday,
267 Wednesday and Friday morning, to allow a sufficient recovery period. Each training session
268 was supervised by an experienced certified physical activity instructor. Heart rates were
269 continuously recorded to reach the expected intensities, based on the pre-screening VO_{2max}
270 values (HR/ VO_2 relationship). For ethical reasons, this program was also proposed to the
271 control group at the study end.

272

273 ***Combined high-intensity interval training and resistance training (HIIT + RT).*** HIIT was
274 always performed before RT to normalize the concurrent training effects (31). The training

275 program was based on the protocol described by Dupuit et al. (11). The HIIT protocol consisted
276 of repeated cycles of sprinting/speeding for 8s followed by slow pedaling (30-40 rpm) for 12s
277 on a WattBike pro Concept2 (with a freewheel and a double air and magnetic braking system).
278 Resistance was low to facilitate acceleration and limit bicycle-wheel inertia. Resistance was
279 controlled to reach ~85% of each participant's HR_{max} during the 20-min session. HR was
280 continuously monitored (A300, Polar, Finland) to control the intensity. Overall, the mean
281 intensity during HIIT sessions corresponded to $89 \pm 5\%$ of HR_{max} . All participants could
282 complete the 20-min exercise program at this intensity after three sessions.

283 Participants learnt the resistance exercise techniques in two training session before the study
284 initiation, to become acquainted with the exercises. Ten repetitions of each exercise with a low
285 load were performed, to adopt the correct body position and range of motion (considering the
286 individual limitations). If a participant could not perform the movement due to a functional
287 limitation or joint pain, another exercise targeting the same muscle group was proposed. In
288 addition, for each exercise requiring loads, the one-repetition maximum (1 RM) indirect
289 method was determined, as previously described (32). The RT program included two different
290 training circuits, inspired by Dupuit et al. (11), with ten exercises performed in the following
291 order: leg press, bench press, knee extension, cable row, dumbbell calf raise, elbow flexion,
292 abdominal muscle, triceps exercises with upper pulley, plank, bum exercises (Circuit 1), and
293 knees extension, pullover, leg press, side raise with dumbbells, dumbbell calf raise, triceps
294 exercises with upper pulley, hip thrust, chin rowing, plank to upright row (Circuit 2).
295 Participants performed a single-set circuit, with a load of 8–12RM. During the training
296 program, the exact percentage of 1RM was not controlled, and for each exercise the set was
297 performed until failure (33), with 60 to 90s rest period between exercises. The workouts were
298 individually supervised by the same certified personal trainer. When participants managed to
299 correctly execute more than 12 repetitions (posture, speed, and range of motion), the load was

300 adjusted to remain in the planned repetition zone. Participants alternated between circuits every
301 3 weeks to minimize boredom and to create some variation in the exercise choice.

302

303 **Statistical analyses.**

304 All statistical analyses were carried out with the STATISTICA version 12.00 software (StatSoft
305 Inc., Tulsa, OK, USA). Data are presented as the mean \pm standard deviation (SD). The data
306 normal distribution was tested using the Kolmogorov–Smirnov test, and the homogeneity of
307 variance was tested with the F-test. Data were log-transformed, when appropriate, before
308 analysis. Two-way analysis of variance (ANOVA) with repeated measures was used to
309 determine group and time effects, and group \times time interactions. When a significant effect was
310 found, post-hoc multiple comparisons were performed using the Newman-Keuls test. The
311 effect size and statistical power were reported when significant main or interaction effects were
312 detected. The effect size was assessed using the partial eta-squared (η^2) and ranked as follows:
313 ~ 0.01 = small effect, ~ 0.06 = moderate effect, ≥ 0.14 = large effect (34). Baseline values and
314 changes (delta value) between baseline and the study end [delta: (12 weeks – baseline /
315 baseline) \times 100] were also compared between groups, using one-way ANOVA. Differences
316 with a P-value ≤ 0.05 were considered statistically significant.

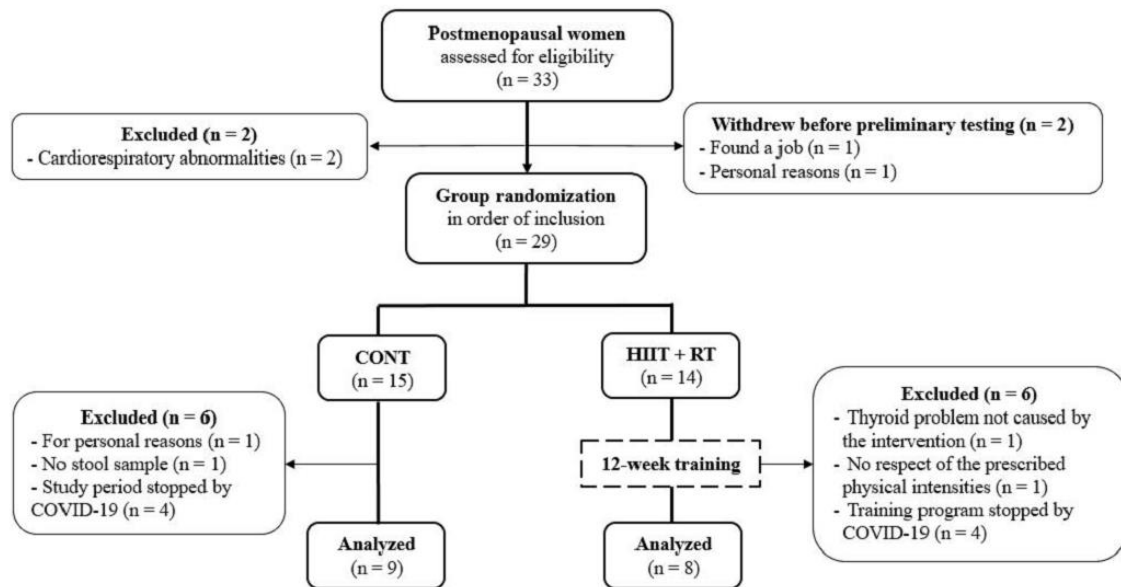
317

318 **RESULTS**

319

320 **Participants' Characteristics.** At the beginning of the protocol, 33 postmenopausal women
321 were eligible (Fig. 1). Then, two were excluded for cardiorespiratory abnormalities, and two
322 withdrew for personal reasons before randomization. Moreover, 12 women did not complete
323 the study (n=6/group). This was explained by the COVID-19-related closure of the training
324 center for 8 women. In total, 17 women completed the study (HIIT + RT: $n = 8$, CONT: $n = 9$,

325 Fig. 1). At baseline, mean age (CONT: 60.9 ± 4.8 years; HIIT + RT: 58.8 ± 5.3 years), total
 326 body weight (HIIT + RT: 78.8 ± 12.5 kg; CONT: 80.3 ± 11.1 kg) and total %FM (HIIT + RT:
 327 35.2 ± 4.9 %; CONT: 33.0 ± 4.6 %) were comparable between groups (Table 1). Participants
 328 attended 97.5% of training sessions and their compliance with the training program was 99%
 329 $\pm 1\%$. No adverse event was reported during testing or training in both groups.



330

331 **Figure 1:** Study flowchart.

332 CONT: control group; HIIT + RT: high-intensity interval training + resistance training.

333

334 **Habitual Energy Intake and Energy Expenditure.** The pre- and post-training physical
 335 activity levels (GPAQ scores) were comparable between groups. The daily energy intake and
 336 the percentage of energy contribution from macronutrients did not significantly change during
 337 the intervention period in each group and was no different between groups (supplementary
 338 table 1).

339

340 **Physical fitness.** Baseline VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$) and Peak Power Output ($Watts \cdot kg^{-1}$) were
 341 not different between groups (Table 1). Overall, the baseline VO_{2max} value ($20.1 \pm 4.6 mL \cdot kg^{-1}$
 342 $\cdot min^{-1}$) indicated a low cardiorespiratory fitness level. After the 12-week intervention, the

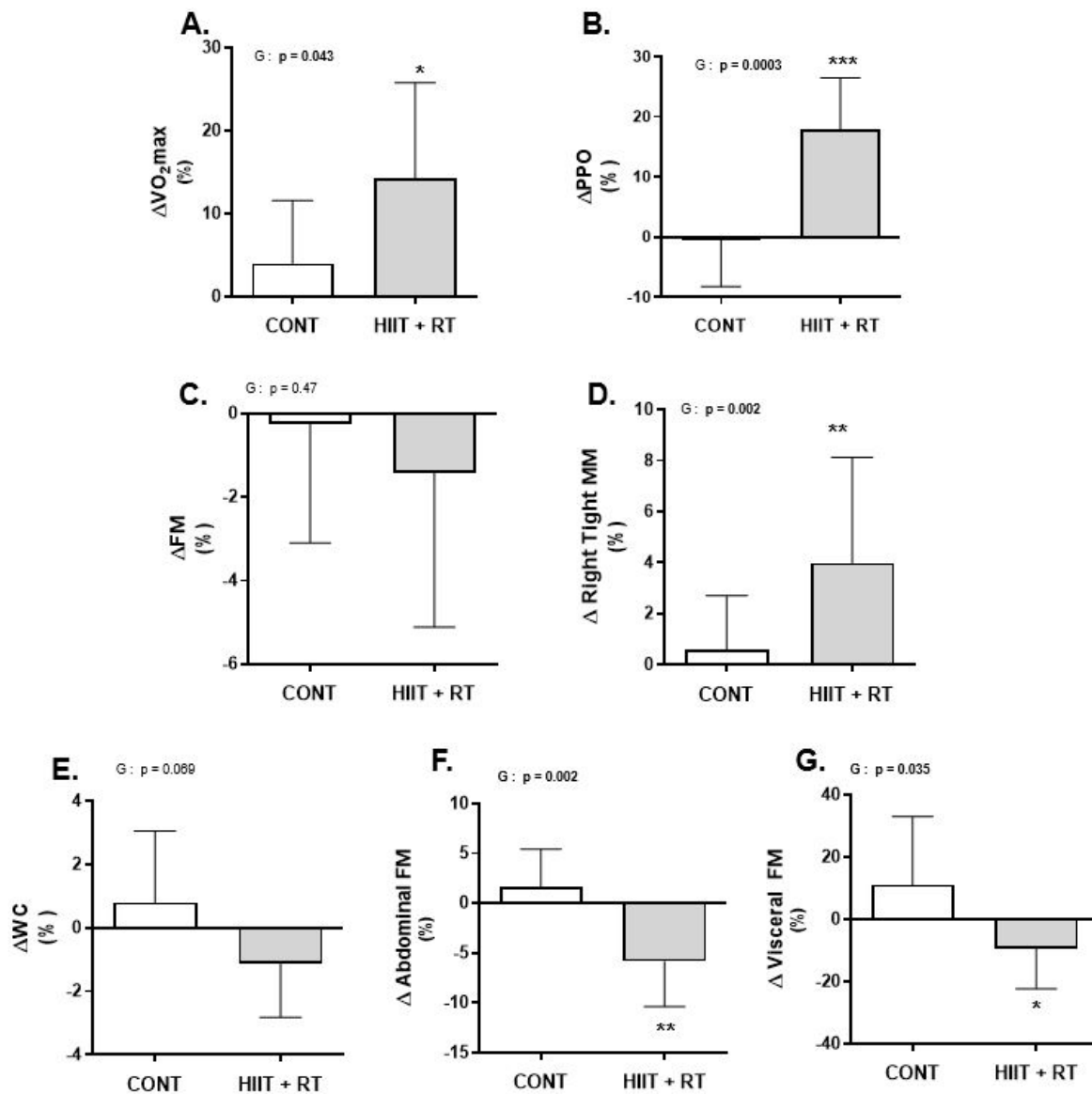
343 VO_{2max} and Peak Power Output (relative values) change were significantly higher in the HIIT
344 + RT than CONT group (+ 14.5 % vs. + 4 % and + 17.8 % vs. - 0.5 %, respectively; $p < 0.05$)
345 (Table 1 and Fig. 2A-B).

346

347 **Anthropometric and body composition measurements.** Baseline body mass and BMI did
348 not differ between groups. Overall, the 12-week intervention induced an increase of these
349 parameters (time effect, $p = 0.047$, $\eta^2 = 0.24$; $p = 0.048$, $\eta^2 = 0.24$, respectively). Total FM (kg)
350 and the percentage of total FM loss did not differ between groups at the study end (Fig. 2C).
351 However, when expressed in percentage of body mass (%BM), total FM was significantly
352 decreased after the 12-week period (time effect, $p = 0.045$, $\eta^2 = 0.24$, Table 1). Waist
353 circumference (described as absolute value and percentage of change) only tended to decrease
354 in the HIIT + RT group ($p = 0.06$ and $p = 0.07$, respectively, with large size effects ($\eta^2 = 0.21$
355 for both) (Table 1 and Fig. 2E). Overall, fat free mass and muscle mass (expressed as kg or
356 %BW) were significantly increased (time effect, $p < 0.05$). However, the right thigh muscle
357 mass was increased only in the HIIT + RT group ($+ 3.99\% \pm 4.15$) at the study end, leading to
358 a significant difference with the CONT group ($p = 0.047$, $\eta^2 = 0.24$, Fig. 2D).

359

360 **Abdominal and visceral fat mass.** Baseline total abdominal (kg) and visceral FM (kg) were
361 similar in the two groups. At the end of the training period, total abdominal FM was
362 significantly reduced only in the HIIT + RT group ($p = 0.007$, $\eta^2 = 0.39$, Table 1) and the
363 percentage of abdominal FM change was significantly different between groups ($p = 0.002$, η^2
364 $= 0.47$, Fig. 2F). The percentage of visceral FM change was negative only in the HIIT + RT
365 group after the intervention ($-7.5\% \pm 10.7$) ($p = 0.035$, $\eta^2 = 0.26$, Fig. 2G). Hence, the HIIT +
366 RT protocol was sufficient to significantly and beneficially impact various anthropometric
367 measurements.



369

370 **Figure 2:** Changes of physical fitness (A-B), body composition (C-D), waist circumference
 371 (E), abdominal (F) and visceral (G) fat mass in the CONT ($n = 9$) and HIIT + RT ($n = 8$) groups
 372 between pre- and post-intervention. Data are the mean \pm SD.

373 CONT: control group; HIIT + RT: high-intensity interval training + resistance training; Δ :
 374 change = $(12 \text{ weeks} - \text{baseline} / \text{baseline}) \times 100$; PPO: peak power output; FM: fat mass; MM:
 375 muscle mass; WC: waist circumference.

376 *: $p \leq 0.05$, **: $p \leq 0.005$, ***: $p \leq 0.0005$: HIIT + RT vs. CONT group.

377

378

379 **Metabolic profile.** The blood parameters at baseline and at the end of the protocol are listed in

380 Table 2. The 12-week intervention did not modify any of the tested metabolic parameters.

	CONT		HIIT + RT		ANOVA (<i>p</i>)		
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>G</i>	η^2 <i>T</i>	<i>G</i> × <i>T</i>
<u>Body composition</u>							
BMI (kg·m⁻²)	31.5 ± 3.4	31.7 ± 3.3	30.3 ± 3.5	30.6 ± 3.5	0.30 0.07	0.048 0.24	0.80 0.00
Body mass (kg)	80.3 ± 11.2	79.3 ± 10.7	77.8 ± 12.4	79.3 ± 12.5	0.78 0.01	0.047 0.24	0.87 0.00
Waist circumference (cm)	102.8 ± 8.6	103.1 ± 10.2	102.1 ± 12.2	101.0 ± 12.3	0.75 0.01	0.82 0.00	0.063 0.21
Total FM (kg)	27.5 ± 7.6	27.0 ± 7.2	28.0 ± 7.4	27.5 ± 6.5	0.47 0.03	0.19 0.11	0.49 0.04
Total FM (%BM)	33.0 ± 4.6	32.2 ± 4.0	35.2 ± 4.9	34.3 ± 4.0	0.29 0.07	0.045 0.24	0.64 0.02
Total FFM (kg)	44.3 ± 3.9	45.4 ± 4.3	50.8 ± 6.5	51.9 ± 7.0	0.73 0.01	0.012 0.35	0.67 0.01
Total FFM (%BM)	54.5 ± 4.6	55.2 ± 4.	64.8 ± 4.9	65.7 ± 4.0	0.29 0.07	0.046 0.24	0.54 0.03
Muscle mass (kg)	42.7 ± 3.7	43.7 ± 4.0	48.8 ± 6.4	49.9 ± 6.8	0.75 0.01	0.013 0.34	0.68 0.01
Muscle mass (%BM)	52.5 ± 4.7	53.2 ± 4.0	62.3 ± 4.8	63.2 ± 3.9	0.33 0.06	0.039 0.26	0.52 0.03
Arm muscle mass (kg)	4.1 ± 0.3	4.0 ± 0.3	4.2 ± 0.6	4.4 ± 0.8*	0.34 0.06	0.59 0.02	0.05 0.23
Tight muscle mass (right side, kg)	5.5 ± 0.6	5.5 ± 0.6	5.4 ± 0.6	5.6 ± 0.6*	0.94 0.00	0.011 0.36	0.043 0.26
Total abdominal FM (kg)	7.2 ± 2.2	7.4 ± 2.2	6.5 ± 1.9	6.0 ± 1.7**	0.33 0.06	0.13 0.15	0.007 0.39

Visceral FM (kg)	4.0 ± 1.5	4.3 ± 1.5	3.2 ± 0.6	2.8 ± 0.5	0.07 0.20	0.53 0.03	0.07 0.19
<u>Physical fitness</u>							
VO₂max (mL·kg⁻¹·min⁻¹)	19.7 ± 3.6	20.5 ± 4.0	20.5 ± 5.7	23.2 ± 5.8**	0.46 0.03	0.001 0.53	0.037 0.26
PPO (Watts)	113 ± 15	114 ± 21	116 ± 26	136 ± 23***	0.24 0.09	≤ 10 ⁻³ 0.68	≤ 10 ⁻³ 0.63
PPO (Watts·kg⁻¹)	1.4 ± 0.3	1.4 ± 0.3	1.5 ± 0.5	1.8 ± 0.5***	0.29 0.07	≤ 10 ⁻³ 0.67	≤ 10 ⁻³ 0.68

381

382 **Table 1: Body composition and physical fitness in the CONT and HIIT + RT groups at baseline (pre) and at the end (post) of the 12-week intervention.**

383 Values are the mean ± SD. G: group effect; T: time effect; G × T: group × time interaction.

384 BMI: body mass index; BM: body mass; FM: fat mass; FFM: free-fat mass; Muscle mass = FFM – Bone Mineral Content by dual-energy X-ray
385 absorptiometry; PPO: peak power output.

386 *: p ≤ 0.05, **: p ≤ 0.005, *** p ≤ 0.005 (pre vs. post in the same group)

387

388

	CONT		HIIT + RT		ANOVA (<i>p</i>)		
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>	<i>G</i>	η^2 <i>T</i>	<i>G</i> × <i>T</i>
Glycemia (mmol·L ⁻¹)	6.39 ± 3.24	6.06 ± 3.26	6.22 ± 1.62	6.44 ± 1.94	0.93 0.00	0.86 0.00	0.34 0.06
Insulinemia (μU·L ⁻¹)	13.24 ± 6.36	10.81 ± 6.12	10.24 ± 2.73	10.17 ± 4.44	0.43 0.06	0.22 0.14	0.44 0.06
HbA1c (%)	6.20 ± 1.64	6.14 ± 1.61	6.20 ± 0.73	6.24 ± 0.65	0.94 0.01	0.83 0.00	0.34 0.06
HOMA-IR	3.76 ± 2.16	2.20 ± 1.99	2.93 ± 1.46	2.99 ± 1.28	0.99 0.00	0.10 0.21	0.22 0.12
Total cholesterol (mmol·L ⁻¹)	5.91 ± 0.88	5.57 ± 0.88	5.97 ± 1.16	5.45 ± 1.23	0.94 0.00	0.07 0.20	0.71 0.01
HDL-C (mmol·L ⁻¹)	1.58 ± 0.17	1.54 ± 0.21	1.49 ± 0.27	1.44 ± 0.25	0.35 0.06	0.35 0.06	0.90 0.00
LDL-C (mmol·L ⁻¹)	3.72 ± 0.76	3.52 ± 0.85	3.84 ± 1.04	3.48 ± 1.02	0.91 0.00	0.10 0.17	0.62 0.02
TG (mmol·L ⁻¹)	1.36 ± 0.46	1.13 ± 0.32	1.39 ± 0.51	1.16 ± 0.38	0.86 0.00	0.09 0.17	0.99 0.00
Total cholesterol/HDL-C	3.77 ± 0.42	3.66 ± 0.68	4.38 ± 1.04	3.81 ± 0.72	0.26 0.08	0.054 0.22	0.18 0.11
usCRP (mg·L ⁻¹)	4.50 ± 2.57	4.48 ± 3.12	2.48 ± 1.70	2.35 ± 1.43	0.14 0.16	0.66 0.02	0.68 0.011

389

390 **Table 2: Metabolic parameters in the CONT and HIIT + RT groups at baseline (Pre) and after (Post) the intervention.**

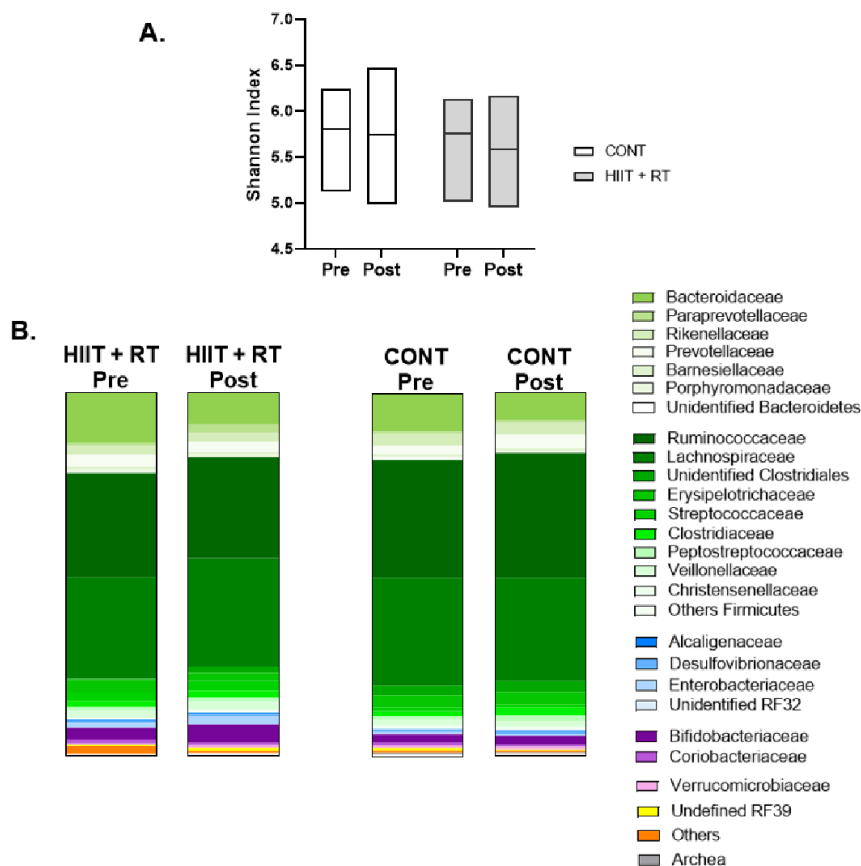
391 Values are the mean ± SD. G: group effect; T: time effect; G × T: group × time interaction.

392 HDL: high density lipoproteins; LDL: low density lipoproteins; C: cholesterol; TG: triglycerides; usCRP: ultrasensitive C reactive protein.

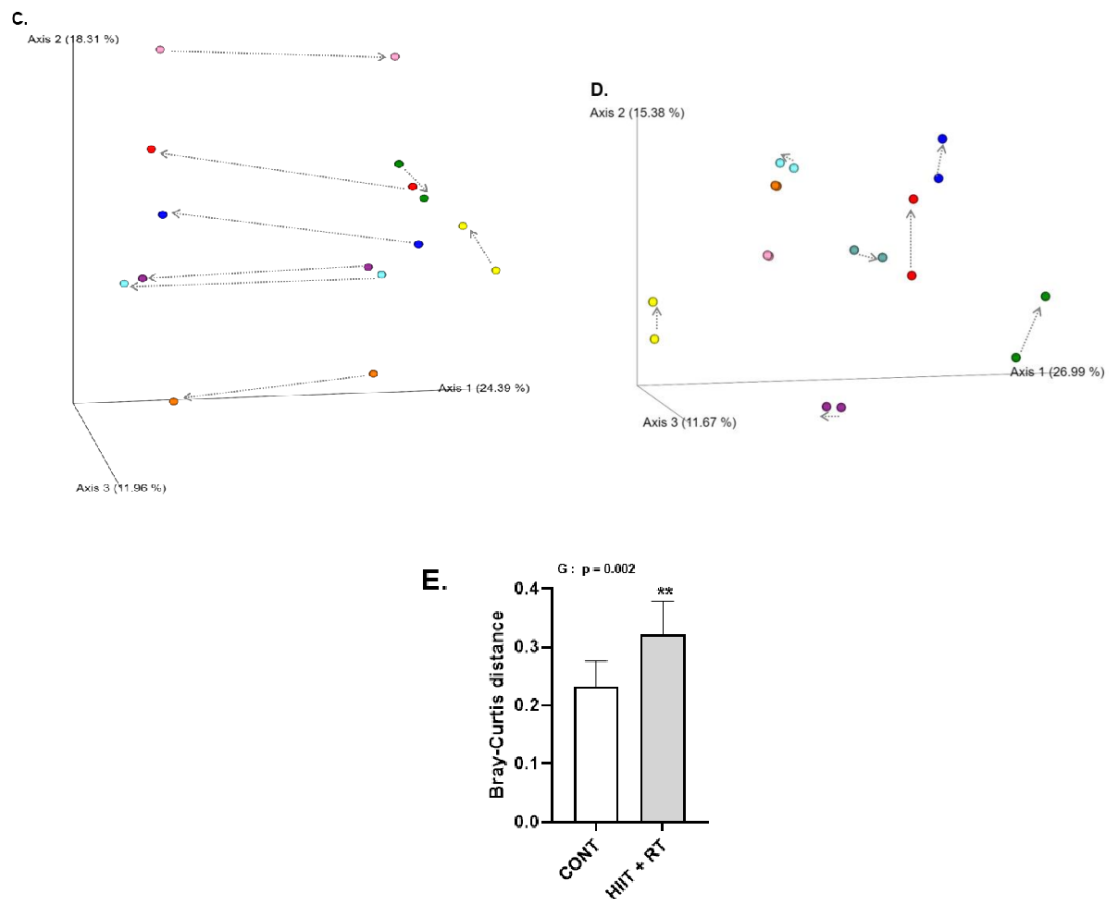
393 **Fecal microbiota composition**

394 Analysis of the fecal microbiota composition by 16S rRNA sequencing revealed that the
 395 baseline α -diversity (Shannon’s diversity index) was not different between groups, and was not
 396 changed at the study end (Fig. 3A). Before and after the intervention, *Firmicutes* and
 397 *Bacteroidetes* were the most abundant phyla in both groups (91.5%). The taxonomy analysis
 398 did not reveal any significant group difference at the phylum and family levels after the 12-
 399 week program (Fig. 3B).

400 On the other hand, β -diversity analysis by PCoA of the unweighted Unifrac distance matrices
 401 showed that the pre- and post-intervention microbiota composition changed in most patients
 402 from the HIIT + RT group (Fig. 3C), whereas it remained stable in the CONT group (Fig. 3D).
 403 This was confirmed by the bigger Bray-Curtis distance measured between pre- and post-
 404 intervention in the HIIT + RT group (Fig. 3E), demonstrating the ability of the HIIT + RT
 405 protocol to modulate fecal microbiota.



406



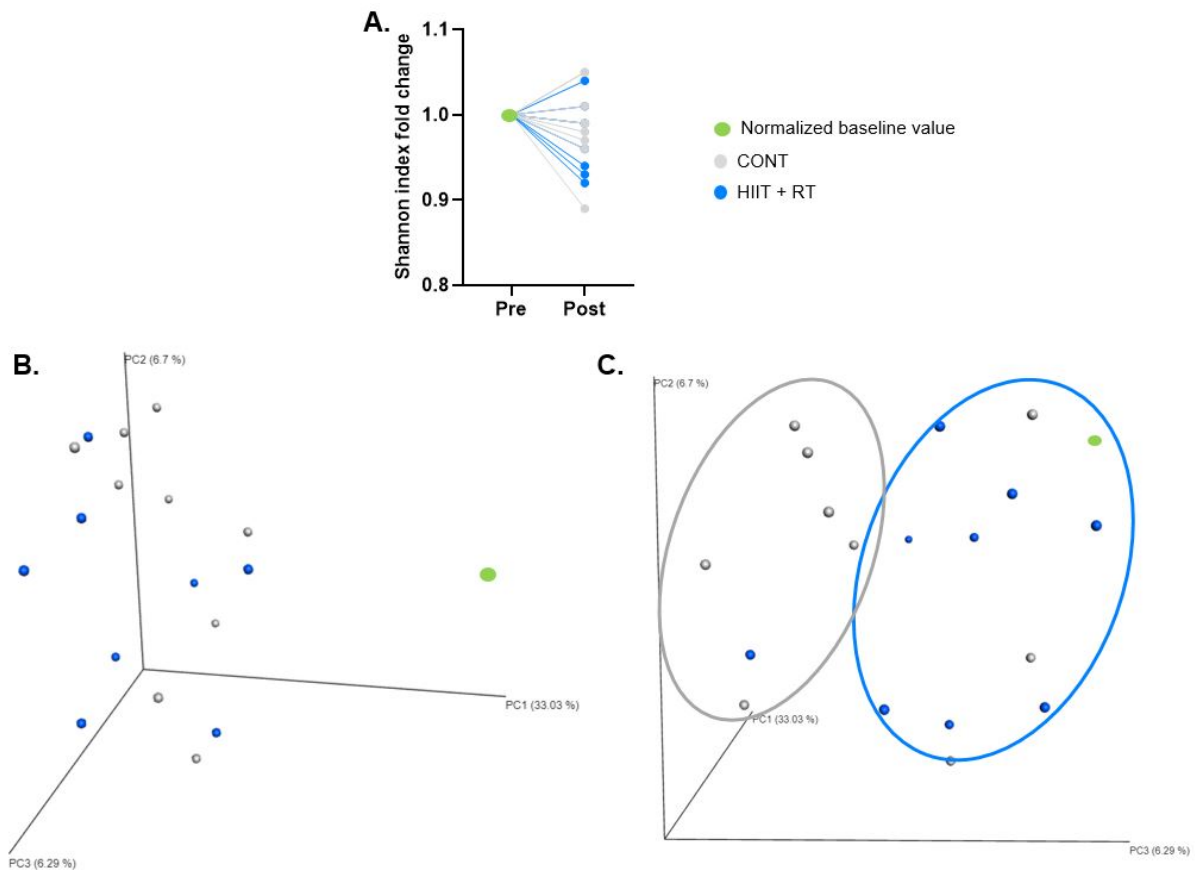
407

408 **Figure 3:** Changes of the α -diversity and the β -diversity in the CONT ($n = 9$) and HIIT + RT
 409 ($n = 8$) groups between baseline (Pre) and study end (Post). (A) Shannon index, (B) Microbiota
 410 composition with the relative abundance of each family, Principal Coordinates Analysis plots
 411 of unweighted Unifrac distance metrics for the HIIT + RT (C) and CONT group (D), and (E)
 412 Bray-Curtis distance between pre- and post-intervention in the HIIT + RT and CONT groups.
 413 CONT: control group; HIIT + RT: high-intensity interval training + resistance training; \rightarrow : pre-
 414 to post-intervention.

415

416 As the PCoA plots (Fig. 3C-D) and the initial fecal microbiota composition (Supplementary
 417 Fig. 1) indicated a high interindividual variability in microbiota composition at baseline, then
 418 microbiota composition for each participant was normalized using their baseline composition
 419 to better highlight composition changes. This approach confirmed that α -diversity variations
 420 were comparable between groups after the intervention (Fig. 4A), and revealed that overall,
 421 fecal microbiota composition was changed in both groups at the study end (Fig. 4B). Moreover,
 422 based on axis 2 and 3, the PCoA plots highlighted clear intervention-based clustering,

423 suggesting specific changes in microbiota composition between trained and untrained women
424 (Fig. 4C).



425

426 **Figure 4:** α - (A) and β -diversity (B-C) changes between baseline (Pre) and study end (Post)
427 using the normalized baseline values for all CONT (gray points, $n = 9$) and HIIT + RT (blue
428 points, $n = 8$) participants.

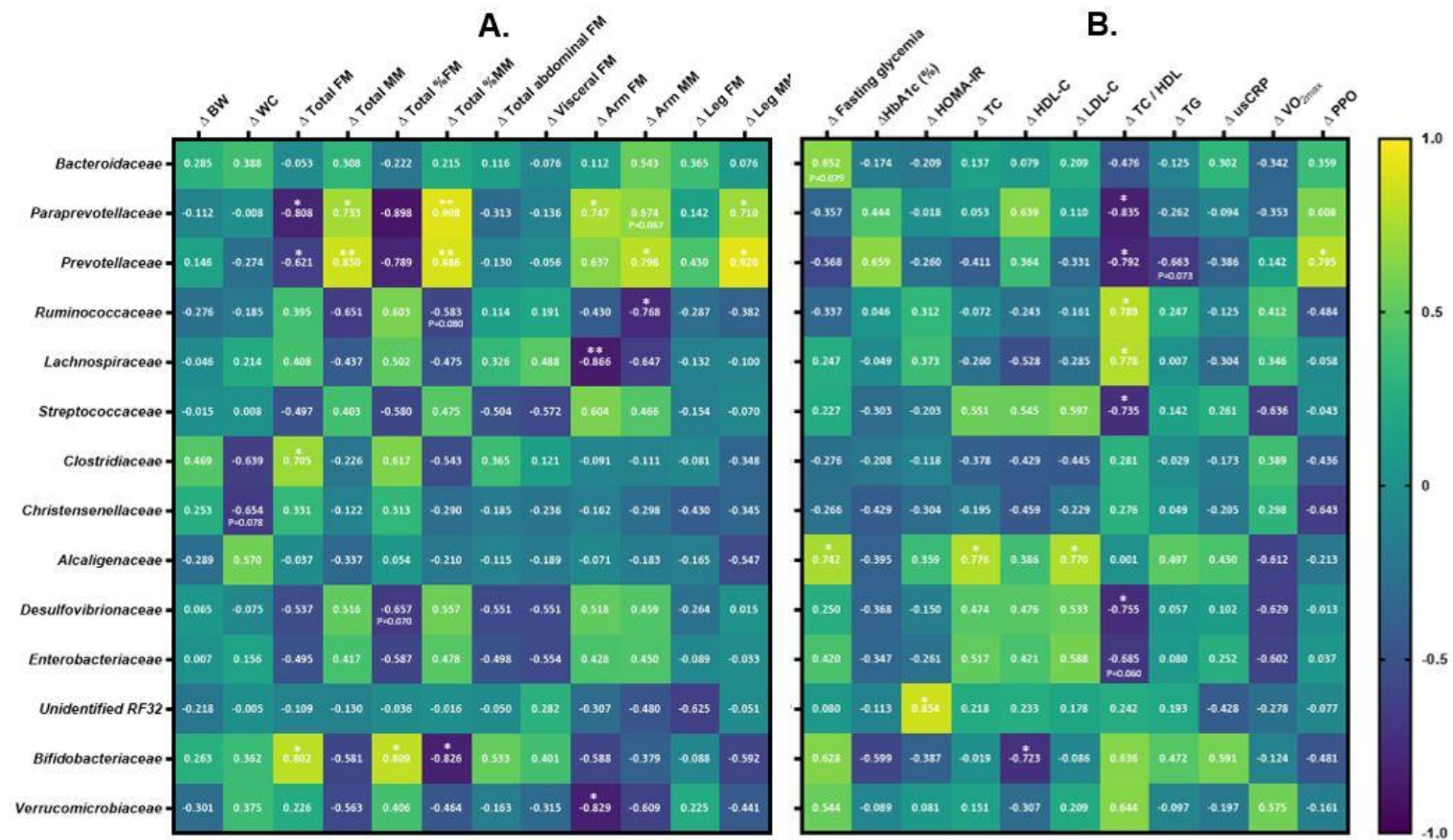
429 CONT: control group; HIIT + RT: high-intensity interval training + resistance training.

430

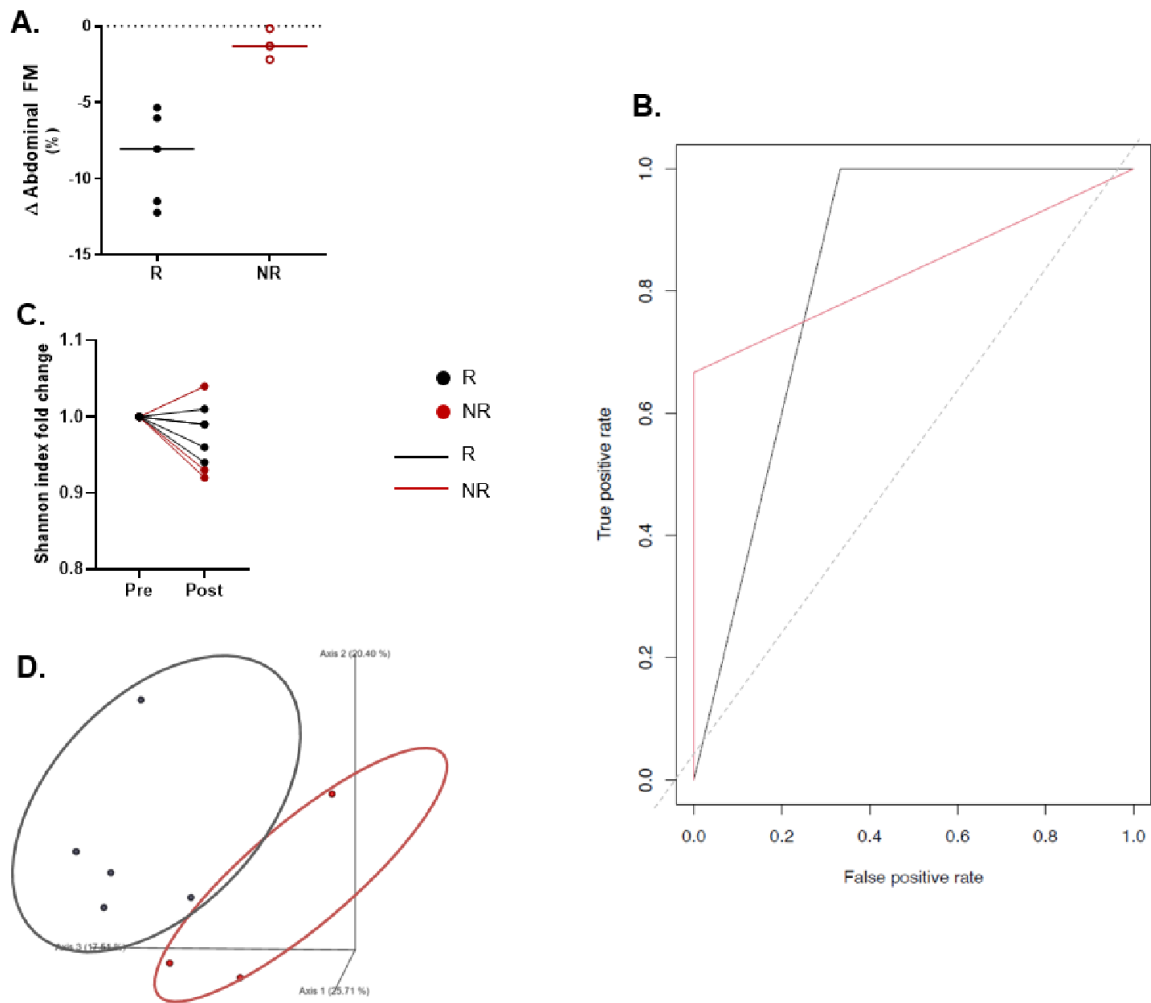
431 Next, a correlation analysis between the baseline relative abundance of specific microbiota
432 families and changes in body composition and cardio-metabolic parameters in the HIIT + RT
433 group (Fig. 5A-B) showed that *Bifidobacteriaceae* abundance was positively correlated with
434 Δ FM and negatively with Δ muscle mass and Δ HDL-C. Conversely, *Paraprevotellaceae* and
435 *Prevotellaceae* were negatively correlated with Δ FM and positively with Δ muscle mass. These
436 two families and *Streptococcaceae* and *Desulfovibrionaceae* were negatively correlated with
437 changes in total cholesterol/HDL-C, unlike *Lachnospiraceae* and *Ruminococcaccae*.
438 *Alcaligenaceae* were positively correlated with fasting glycemia, total cholesterol and LDL-C

439 changes. *Prevotellaceae* was also positively correlated with PPO changes, and
440 *Christensenellaceae* relative abundance tended to be negatively correlated with changes in
441 WC. The Shannon's index was positively correlated with VO_{2max} changes (Fig. 5C), suggesting
442 an association between rich microbiota and cardiorespiratory fitness improvements.

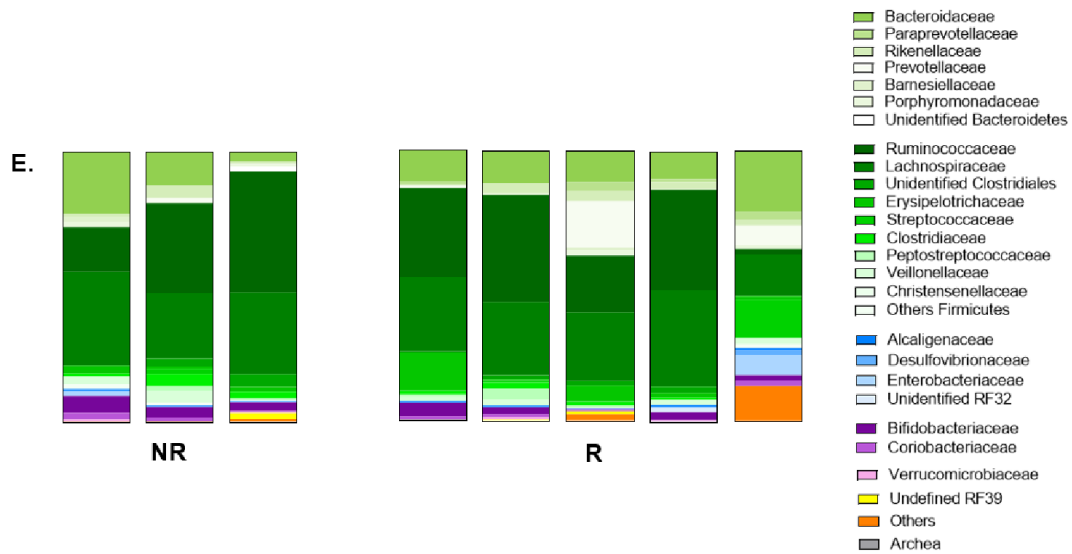
443 As complementary observational data, to determine whether the training outcomes were related
444 to a specific baseline microbiota composition, HIIT + RT participants were grouped into
445 responders (R, $n = 3$) and non-responders (NR, $n = 5$), based on the post-intervention total
446 abdominal FM loss (cut-off: -2.5%; Fig. 6A). Then, a random forest algorithm that integrated
447 the baseline microbial features was used to investigate the ability of baseline microbiota
448 composition to predict the response to the HIIT + RT protocol. The obtained ROC curves (Fig.
449 6B) had an AUC of 0.83, indicating a relatively good ability of microbiota composition to
450 predict future HIIT + RT efficacy. Although the Shannon's diversity index did not show any
451 difference between R and NR when expressed as fold change (Fig. 6C), two clusters were
452 observed when using the β -diversity values that separated the R and NR participants, further
453 suggesting that the baseline microbiota composition might predict the response to HIIT + RT
454 (Fig. 6D-E).



456 **Figure 5:** Associations between baseline relative abundance of specific microbiota families
 457 and body composition (A) and cardio-metabolic parameters (B); and between α -diversity and
 458 physical fitness (C) in the HIIT + RT group.
 459 Δ : change between baseline and intervention end (%), BW: body weight; FM: fat mass; MM:
 460 muscle mass; WC: waist circumference; TC: total cholesterol; HDL: high density lipoproteins;
 461 LDL: low density lipoproteins; C: cholesterol; TG: triglycerides; usCRP: ultrasensitive reactive
 462 C protein; PPO: peak power output.
 463 * $p < 0.05$, ** $p < 0.005$, *** $p < 0.005$.
 464



465



466

467 **Figure 6:** Abdominal fat mass (FM) changes (%) between baseline and study end in
 468 participants from the HIIT + RT group divided in responders (R) and non-responders (NR) (A).
 469 ROC curve representing the ability of microbiota composition to predict the response to
 470 training in R and NR (B). α - and β -diversity analysis in R and NR (C-D). Individual basal
 471 microbiota composition at the family level in R and NR (E).

472

473

474 DISCUSSION

475

476 The aim of this study was to determine the impact of a 3-month HIIT + RT program on
 477 body composition and fecal microbiota in non-dieting postmenopausal women with overweight
 478 or obesity. Compared with the control group, the HIIT + RT program significantly increased
 479 physical fitness (VO_{2max} , Peak Power Output), decreased total abdominal and visceral FM, and
 480 enhanced segmental muscle mass. The fecal microbiota β -diversity significantly changed
 481 between pre- and post-intervention in the HIIT + RT group, and the Shannon's index was
 482 positively correlated with VO_{2max} changes. Notably, various intestinal microbiota components
 483 correlated with HIIT + RT-induced body composition changes, and baseline microbiota
 484 composition predicted the response to the HIIT + RT program.

485

486 In women, menopause is associated with body composition modifications including FM
487 gain, particularly (intra-)abdominal adipose tissue, and muscle mass loss (35). FM
488 accumulation and its adverse distribution (*i.e.* abdominal/visceral deposits) partly explain the
489 higher CVD risks in this population (7, 8). Regular physical activity might be an efficient
490 strategy to prevent and counteract estrogen deficiency-induced FM gain and abdominal FM
491 deposit in postmenopausal women (35). However, the frequency of spontaneous physical
492 activity seems to be lower in post- than in pre-menopausal women (6). In accordance with
493 several reviews and meta-analyses (36, 37), our group demonstrated that HIIT is a safe and
494 time-efficient strategy to reduce total and (intra-)abdominal FM in pre- and postmenopausal
495 women, like in men (38, 39). Recently, our laboratory also showed that compared with HIIT
496 alone, the HIIT + RT combination more effectively decreases (intra-)abdominal FM (kg and
497 %) and increases total muscle mass (%) in postmenopausal women with overweight/obesity
498 (11). Using the same protocol [60 × 8s at 80-90% of HR_{max} , 12s active recovery + 10 whole-
499 body resistance exercises: 1 set of 8-12 repetitions], we obtained similar results in the present
500 study, indicating a large effect of HIIT + RT on total abdominal and visceral FM loss. Similarly,
501 Rashti et al. observed a decrease in total abdominal FM (both visceral and subcutaneous
502 adipose tissue assessed by MRI) after 10 weeks of HIIT (4 × (4' 85-95% HR_{max} / 4' 65% HR_{max})
503 + RT (3-4 × 8-15 repetitions $r = 30-60s$ and $R=2-3'$) in active and non-active postmenopausal
504 women (45-65 years) (12). However, HIIT and RT were not performed in the same session.
505 Conversely, in older women (60-70 years), Yoon et al. did not observe any change in total and
506 abdominal FM when the training included first RT (9 exercises) and then HIIT (10 × 30s at 80-
507 90% $HR_{reserve}$ / 90s 50-60% $HR_{reserve}$) in the same session, suggesting that the order of the
508 training modalities may differently affect FM (40). A possible reason of these discrepancies is
509 suggested in the review by Methenitis (31) showing that in concurrent training, the intra-

510 session exercise sequence is important because the first pathway to be activated could inhibit
511 the molecular adaptations induced by the second exercise mode. Concerning fat free mass, our
512 study showed an increase of segmental muscle mass (kg), corresponding to the training-
513 solicited muscle groups. This muscle gain might enhance resting metabolic rate and therefore,
514 the 24-h energy expenditure, favoring FM loss as part of the energy provided through lipid
515 oxidation. As physical activity levels and total energy intakes remained unchanged during the
516 study, our findings reinforces our conclusion that HIIT + RT on its own is an efficient strategy
517 to modify body composition in postmenopausal women with overweight/obesity. The normal
518 basal plasma metabolic values and HOMA-IR in both groups, despite overweight/obesity,
519 might explain why the concurrent training failed to improve concomitantly the lipid profile and
520 glucose homeostasis.

521 The fitness level of the postmenopausal women included in our study was evaluated
522 using the VO_{2max} and PPO values. Lower cardiorespiratory fitness is associated with high BMI
523 and increased CVD risks (41). Moreover, the increase in total and (intra-)abdominal adiposity
524 that occurs after menopause is associated with lower VO_{2max} (42). Our training program
525 significantly improved VO_{2max} and PPO. In the training group, VO_{2max} improvement was in the
526 same range (~15%) as what was observed in postmenopausal women in the study by Dupuit et
527 al. using exactly the same HIIT + RT protocol (11).

528 An increasing body of evidence suggests that gut microbiota can be rebalanced by exercise
529 (15–17). Indeed, many studies reported that physical activity increases the number of beneficial
530 microbial species, enriches microbiota diversity, and improves the development of commensal
531 bacteria leading to health benefits (15). Lower microbiota diversity and higher
532 *Firmicutes/Bacteroidetes* ratio have been associated with obesity, type 2 diabetes and impaired
533 blood glucose (43). Our study was the first to examine the effect of a HIIT + RT program on
534 microbiota composition in postmenopausal women. Our training intervention did not

535 significantly change the α -diversity and overall taxonomy of the fecal microbiota, but
536 modulated the β -diversity. In humans, the absence of training effect on the α -diversity is quite
537 common, whereas the results on the β -diversity are more controversial (44). According to
538 Shahar et al. 2020, physical activity-associated changes in β -diversity could be explained also
539 by physical training-induced modifications of mitochondrial physiology and not only be the
540 direct effects of exercise on the gastrointestinal tract. Mitochondrial physiology would not have
541 the similar effect on α -diversity (*i.e.* the microbiota community richness and evenness) (44).
542 The mixed results concerning the effect of training on β -diversity (increase or not significant
543 changes) may be partly ascribed also to the confounding effect of various factors, such as sex,
544 age, diet, metabolic profile, fitness level and physical activity modality (duration, frequency
545 and modes), that may also influence gut microbiota composition. Furthermore, the different
546 collection and analysis techniques of the fecal microbiota may also bias comparisons (45).
547 When studies using physical activity programs are compared, some authors did not find any β -
548 diversity difference (23, 46–48), whereas others detected significant changes (21, 22). Our
549 results support the ability of HIIT + RT to modulate fecal microbiota in a population of
550 postmenopausal women with overweight or obesity. Similarly, Zhong et al. recently showed
551 that a 2-month program (4 d.wk⁻¹) of combined aerobic (steps: 4 × 4' / r = 20s, undefined
552 intensity) and RT (elastic band: 2-3 × 8-15 repetitions) modifies the gut microbiota β -diversity
553 in postmenopausal women (60-75 years) (49). Besides the classical richness and diversity,
554 physical training may also modulate the relative abundance of specific phyla, families or
555 bacterial species (20, 23, 48). We did not detect such an effect. This could be due to the
556 relatively small number of participants in our study. However, it is also worth noting that
557 although the human gut microbiota is relatively stable at the phylum level, the microbial species
558 and subspecies and their proportions may be specific to each person. In other words, human
559 studies are characterized by a large heterogeneity of the gut/fecal microbiota (50). To overcome

560 such difficulties, we normalized basal microbiota data in the two groups to better detect the
561 potential effects of training. This strategy demonstrated that training may specifically modulate
562 gut microbiota, as shown by the two distinct clusters in the PCoA plot. Moreover, to establish
563 the association between baseline fecal microbiota composition and training-induced body
564 composition and metabolic profile changes, we performed correlation analyses. Contrasting
565 findings are observed in the literature regarding the effect of exercise interventions on fecal
566 counts for the phyla which make comparisons difficult. Our analyses showed that
567 *Bifidobacteriaceae* abundance was positively correlated with FM change. This result has been
568 already shown by Munuka et al. in sedentary overweight women after six weeks of endurance
569 training (21). *Bifidobacteriaceae* metabolize glucose and produce lactic acid and acetic acid
570 (51) that regulates GPR41 and GPR43 (52), two short chain free fatty acid receptors with
571 possibly a protective role against obesity. Conversely, *Paraprevotellaceae* and *Prevotellaceae*
572 were negatively correlated with FM change (%) and positively associated muscle mass change
573 (%). Interestingly, the Shannon's index was positively correlated with $\Delta\text{VO}_{2\text{max}}$, suggesting an
574 association between rich microbiota and cardiorespiratory fitness improvements. This confirms
575 the findings by Estaki et al. showing a significant relationship between fecal microbiota
576 diversity and $\text{VO}_{2\text{max}}$ (53). On the other hand, Bycura et al. did not find any difference between
577 pre-intervention microbiota composition and cardiorespiratory fitness changes after 8 weeks of
578 cycling endurance training, free aerobic activities or resistance training (24).

579 Some people do not respond favorably to exercise. The term 'non-responder' is often
580 used to describe the lack of response (to an exercise intervention) for a pre-specified outcome.
581 As the main outcome of our study included (intra)-abdominal FM loss, we separated arbitrarily
582 our training group in "responders" and "non-responders" based on the post-intervention total
583 abdominal FM loss (-2.5% cut-off). By using a random forest algorithm integrating baseline
584 microbial features, our results showed that baseline microbiota composition can predict the

585 response to the HIIT + RT protocol, thus strengthening the link between fecal microbiota
586 composition and exercise-induced body composition changes.

587 One of the limitations of this study concerns the small number of participants. Indeed,
588 our sample was sufficient to highlight, as expected, a significant (intra-)abdominal FM loss in
589 the HIIT + RT group, but the high inter-individual variability in fecal microbiota composition
590 made difficult the determination of the potential cross-talk between gut microbiota and adipose
591 tissue after the training program (54). However, the normalization of the baseline fecal
592 microbiota composition values and the analysis made in responders and non-responders
593 strengthen our hypothesis. Another limitation was the lack of diet monitoring throughout the
594 intervention period. Diet was recorded using a 5-day food-intake diary only at baseline and at
595 week 12. We cannot guarantee that the diet did not fluctuate between these time points and/or
596 that specific components were not included in the diet at some point, thus contributing to
597 modulate gut microbiota composition (55).

598

599 Our study was the first to assess the impact of a HIIT + RT program on fecal microbiota
600 in postmenopausal women with overweight/obesity. We confirmed that a 12-week cycling
601 HIIT + RT program decreases (intra-) abdominal FM, increases active muscle mass, and
602 improves cardiorespiratory fitness in this population. These modifications were partly related
603 to the exercise-induced modulation of the fecal microbiota. In addition, results of the current
604 study also suggest that baseline gut microbiota composition might predict HIIT + RT
605 efficiency. These findings must be confirmed in a larger sample, but could have potential
606 implications for obesity management, both for physical activity professionals and also for
607 nutritionists/dieticians who can modulate gut microbiota through the diet or specific dietary
608 supplements.

609

610

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615

616 **Conflict of interest**

617 The results of this study are presented clearly, honestly, and without fabrication, falsification,
618 or inappropriate data manipulation. The results of the present study do not constitute
619 endorsement by the American College of Sports Medicine.

620

621 **Competing interests**

622 The authors declare that they have no competing interests.

623

624 **Authors' contributions**

625 MD was the PhD student in the PACWOMan study and designed and supervised the different
626 training modalities. She met all participants, carried out the anthropometric measurements,
627 collected and analyzed all data, supervised training sessions, processed fecal samples, and
628 wrote the first and subsequent drafts of the paper. MR was a co-investigator, and assisted with
629 the study design. CM and PB, physicians, assisted with the study design, and oversaw the
630 medical aspects of the study. MD supervised training sessions and analyzed dietary. MD
631 extracted DNA of fecal sample and BC analyzed microbiota by 16S rRNA gene sequencing
632 using Illumina technology. NB conceived the study idea, was responsible for the overall study
633 design, and for monitoring data collection. All authors read and approved the final manuscript.

634

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644 the decision to submit the article for publication.

645

646

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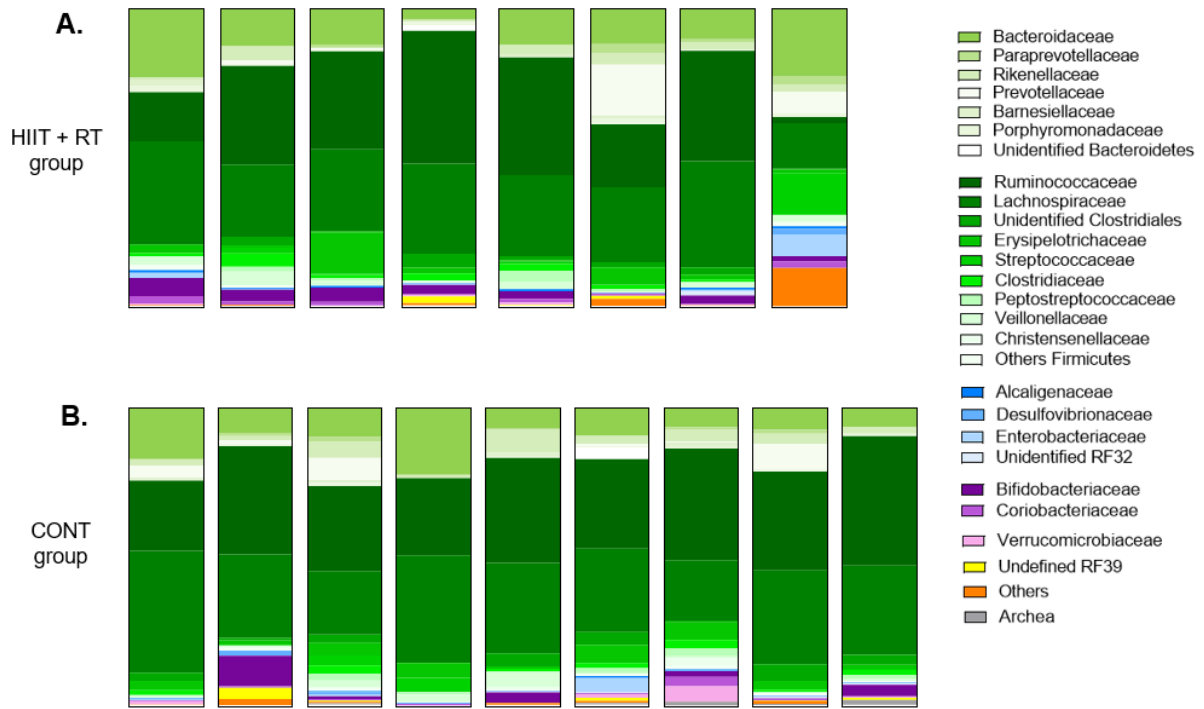
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782 **Supplementary Figure 1:** Individual basal microbiota composition at the family level in the
 783 HIIT + RT ($n = 8$) (A) and CONT ($n = 9$) (B) groups.
 784 CONT: control group; HIIT + RT: high-intensity interval training + resistance training.
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788 **Supplementary Table 1:** Mean daily energy intake and macronutrient repartition in the CONT
 789 and HIIT + RT groups at baseline (pre) and at the end (post) of the 12-week intervention.
 790 TEI: Total energy intake; CHO: carbohydrates; CONT: control group; HIIT + RT: high-
 791 intensity interval training + resistance training.

	CONT		HIIT + RT	
	<i>Pre</i>	<i>Post</i>	<i>Pre</i>	<i>Post</i>
Energy intake (kcal)	1608 ± 287	1682 ± 366	1551 ± 239	1447 ± 251
CHO (%TEI)	37 ± 7	36 ± 7	43 ± 7	40 ± 7
Fat (%TEI)	38 ± 5	40 ± 7	35 ± 7	38 ± 4
Protein (%TEI)	20 ± 5	18 ± 3	18 ± 4	19 ± 3

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