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Annaëlle Couvert, Leslie Goumy, Florie Maillard, Alexandre Esbrat, Kassandra Lanchais, et al.. Effects of a Cycling versus Running HIIT Program on Fat Mass Loss and Gut Microbiota Composition in Men with Overweight/Obesity. *Medicine and Science in Sports and Exercise*, 2024, 56 (5), pp.839-850. 10.1249/MSS.0000000000003376 . hal-04402695

HAL Id: hal-04402695

<https://hal.science/hal-04402695v1>

Submitted on 18 Jan 2024

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Accepted for Publication: 13 December 2023

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Conflict of Interest and Funding Source: This work was supported by the “Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (MESRI), Clermont Auvergne University (AME2P laboratory), Inserm (Institut national de la santé et de la recherche médicale; UMR1071), INRAE (Institut national de recherche en agriculture, alimentation et environnement; USC 1382), the Agence Nationale de la Recherche of the French government through the program “Investissements d'Avenir” (16-IDEX-0001 I-SITE project CAP 20-25 project) of Clermont Auvergne University. BC's laboratory is supported by a Starting Grant from the European Research Council (ERC) under the European Union Horizon 2020 research and innovation program (grant agreement No. ERC-2018-StG- 804135), an award from the Fondation de l'avenir (AP-RM-21-032), the ANR grants EMULBIONT (ANR-21-CE15-0042-01) and DREAM (ANR-20-PAMR-0002) and the national program “Microbiote” from INSERM.

ABSTRACT

Purpose: High-intensity interval training (HIIT) can efficiently decrease total and (intra-) abdominal fat mass (FM); however, the effects of running versus cycling HIIT programs on FM reduction have not been compared yet. Additionally, the link between HIIT-induced FM reduction and gut microbiota must be better investigated. The aim of this study was to compare the effects of two 12-week HIIT isoenergetic programs (cycling vs. running) on body composition and fecal microbiota composition in nondieting men with overweight or obesity.

Methods: Sixteen men (age: 54.2 ± 9.6 years; BMI: 29.9 ± 2.3 kg.m⁻²) were randomly assigned to the HIIT-BIKE (10x45s at 80-85% of HRmax, 90s active recovery) or HIIT-RUN (9x45s at 80-85% of HRmax, 90s active recovery) group (3 times per week). DXA was used to determine body composition. Pre- and post-intervention fecal microbiota composition was analyzed by 16S rRNA gene sequencing, and diet was controlled.

Results: Overall, body weight, abdominal and visceral FM decreased over time ($p < 0.05$). No difference was observed for weight, total body FM and visceral FM between groups (% change). Conversely, abdominal FM loss was greater in the HIIT-RUN group (-16.1% vs -8.3%; $p = 0.050$). The α -diversity of gut microbiota did not vary between baseline and intervention end and between groups, but was associated with abdominal FM change ($r = -0.6$; $p = 0.02$). The baseline microbiota profile and composition changes were correlated with total and abdominal/visceral FM losses.

Conclusions: Both cycling and running isoenergetic HIIT programs improved body composition in men with overweight/obesity. Baseline intestinal microbiota composition and its post-intervention variations were correlated with FM reduction, strengthening the possible link between these parameters. The mechanisms underlying the greater abdominal FM loss in the HIIT-RUN group require additional investigations.

Key Words: HIGH-INTENSITY INTERVAL TRAINING, CYCLING, RUNNING, BODY COMPOSITION, GUT MICROBIOTA, HEALTH

ACCEPTED

INTRODUCTION

Obesity is a complex disease that is primarily driven by sedentary lifestyles, low physical activity, and high-calorie or unbalanced diets and that promotes chronic diseases and disability. Excess fat mass (FM) and metabolic disturbances are associated with higher prevalence of cardiovascular diseases (CVD), type 2 diabetes and many cancer types (1,2). Abdominal and more specifically intra-abdominal (*i.e.* visceral) FM is a metabolically active adipose depot that is strongly associated with obesity-related complications (3). Reducing (intra-)abdominal FM decreases the CVD risk (4). Dietary intervention, lifestyle changes, exercise, medications and their combination are considered relevant means to fight overweight and obesity.

Regular exercise decreases FM and simultaneously increases the cardiorespiratory capacity and preserves the lean mass (5). Research is still needed to identify the best physical activity programs for improving body composition in individuals with overweight or obesity. In the last ten years, high-intensity interval training (HIIT), which includes repeated bouts of high-intensity efforts followed by recovery, has become a popular exercise type to prevent and treat obesity-related conditions. Indeed, it is considered a time-efficient strategy to decrease FM deposits, including total abdominal FM (*i.e.* subcutaneous abdominal FM and visceral adipose tissues) (6–10). However, the exercise modality (running *vs.* cycling) might influence the results. In their meta-analysis of data on adults with normal weight and overweight/obesity, Maillard et al. (2018) reported that running (*vs.* cycling) is more effective in reducing total body FM and visceral adipose tissues, while cycling is better for decreasing total abdominal FM. However, most studies used running and cycling HIIT protocols with different workout intensities (9) and very few studies directly compared the effects of isoenergetic running and cycling HIIT

programs. Yet, after acute high-intensity interval exercise of equivalent energy expenditure, the magnitude of excess post-exercise oxygen consumption (EPOC) is larger in people who performed running than cycling (11). Moreover, plasma lactate concentrations are higher in cycling reflecting a greater carbohydrate utilization (11). Thus, overall, the lower muscle mass involved in cycling (12), differences in muscle contraction regimens (*i.e.*, concentric vs. eccentric), greater mechanical efficiency in running due to the stretch-shortening cycle (13), and differences in catecholamine production at the same relative intensity could explain the total and/or regional fat oxidation differences observed between these modalities (9,14) and could lead to different FM losses.

In addition, obesity development is increased by an unfavorable balance of the intestinal microbiota, known as dysbiosis. It is widely recognized that the intestinal ecosystem plays, through direct or indirect mechanisms, a significant role in causing systemic inflammation, insulin resistance, and body composition alterations (15). Studies in humans and animal models showed that HIIT, like any chronic physical activity, can alter the intestinal microbiota composition and that these changes are associated with body composition changes (7,16,17). However, until now, no study has directly compared this potential association using isoenergetic running and cycling HIIT programs.

Based on these data and to support public health recommendations on the best exercise modalities, the aim of this study was to compare the effectiveness of two 12-week isoenergetic HIIT programs (running *vs* cycling) on total body and (intra-)abdominal FM loss in men with overweight or obesity, and to evaluate the implication of gut microbiota changes. We

hypothesized that both programs would be effective in decreasing FM deposits, including total and (intra)-abdominal FM, and that the running HIIT program would induce larger effects despite similar gut microbiota composition alterations.

METHODS

On the basis of our previous results on visceral FM loss after a 3-month HIIT + resistance training program (6), the sample size was determined before the study starts to ensure a statistical power of 80%. Considering a two-sided type I error at 5%, a minimal difference of 1.5 kg in visceral FM loss (SD = 1.0) could be detected with seven participants per group. The sample size was increased to ten participants per group to take into account individuals lost to follow-up.

This study was approved by the relevant ethics committee (Comité de Protection des Personnes Ouest Est-II, CPP 19.11.29.46256) and was registered on ClinicalTrials.gov (ClinicalTrials.gov: NCT05311800). Participants were recruited via flyers, posters and advertisements on websites and social networks. Before inclusion, participants were given explanations on the study aims and methods and their written informed consent was collected.

Participants

For practical and feasibility reasons (mostly related to the COVID-19 pandemic) the study was carried out in three waves from February 2020 and July 2022. Twenty participants were recruited according to the following criteria: adult (18-65 years of age) men, body mass index (BMI) ≥ 25 kg/m² and ≤ 35 kg/m², stable body weight for at least 3 months, stable eating habits and physical activity for at least 3 months. The non-inclusion criteria were: medical

contraindications to intense physical activity, painful joints, medical or surgical history judged incompatible with the study, treatment with beta-blockers or any other drug that could interfere with the study, any specific dietary pattern (e.g. vegan), and probiotic or antibiotic consumption in the last 3 months. Twenty participants were enrolled through a rolling recruitment process, and each man was alternately assigned to the HIIT-RUN or HIIT-BIKE group. In total, 16 participants completed the study [HIIT-RUN (n = 8), HIIT-BIKE (n = 8)] (Figure 1). All participants reported low levels of physical activity, based on the Global Physical Activity Questionnaire (GPAQ) results (18). None of them had history of chronic arterial or respiratory diseases, CVD, or endocrine disorders. Participants were given at least 10 days to become familiar with the equipment (treadmill and bicycle) before the protocol start.

Experimental design

Anthropometric and body composition measurements. Body weight was measured to the nearest 0.1 kg on a Seca 709 scale (Balance Seca 709, France) in fasting conditions. Height was measured to the nearest 0.5 cm with a wall-mounted stadiometer. BMI was calculated as body weight (kg) divided by the square of height (m²). Using a measuring tape and in supine position, waist circumference (cm) was measured at the edge of the upper iliac crests (cm) and hip circumference at the level of the femoral trochanters. The sagittal abdominal diameter (supine abdominal height) was measured with a Holtain–Kahn abdominal caliper (Holtain Limited, Crymych, Pembrokeshire, UK) to the nearest mm in the sagittal plane at the level of the iliac crests (L4–L5) in participants lying supine on a firm bench with bent knees during normal expiration. Abdominal skinfold thickness was measured at four different sites (at 15 cm and 7 cm to the right and left of the navel) with a Harpenden Skinfold Caliper (Mediflex Corp., Long Island, NY,

USA), and the mean subcutaneous abdominal skinfold thickness was then calculated (19). The same experienced investigator took all anthropometric measurements at baseline and after 12 weeks of training.

Fat and fat-free mass localization. Whole-body mass and regional FM as well as fat-free mass (FFM) (expressed as kg and % of body mass) were measured using a dual-energy X-ray absorptiometry (DXA) scan (QDR-4500A, Hologic, Inc., Marlborough, MA, USA). Two regions of interest were manually isolated and analyzed by an experienced technician: the area from L1–L2 to the pubic rami (to calculate total abdominal FM) and the area from the iliac crest to the feet (to calculate the lower body FM). The same operator performed all analyses. Total visceral FM (kg) was estimated from the mean subcutaneous abdominal skinfold thickness, abdominal height and total abdominal FM (DXA), as previously described (20).

Preliminary visit - maximal exercise testing. Maximum oxygen consumption (VO_{2max} expressed in $ml \cdot min^{-1} \cdot kg^{-1}$ and $ml \cdot min^{-1} \cdot kg \text{ FFM}^{-1}$) was measured during an incremental test on a cycle ergometer (General Electric T2100) or on a treadmill (Ergoline, Bitz, Germany) depending on the participants' group.

For the cycling condition, participants were asked to pedal at a constant speed of 60-70 rpm, but at increasing intensities (steps of 1 minute each) until they reached the VO_{2max} . The increment (15-20W) was chosen by the physician according to the participant's age and was identical, for that participant, from the beginning to the end of the training program. For the running condition, the treadmill test started at $4 \text{ km} \cdot h^{-1}$, then the speed was increased by $1 \text{ km} \cdot h^{-1}$

per minute, at a constant slope of 1% until the $\text{VO}_{2\text{max}}$ was reached. Gas exchanges [oxygen consumed (VO_2) and carbon dioxide released (VCO_2)] were measured breath-by-breath using a respiratory mask connected to a gas analyzer (Oxycon pro-Delta, Jaeger, Hoechberg, Germany). $\text{VO}_{2\text{max}}$ was defined as the maximum oxygen consumption averaged over a period of 15 seconds. Ventilatory parameters were averaged every 30 seconds. Heart electrical activity was recorded with an electrocardiogram (ECG) throughout the test. Participants were verbally encouraged by the experimenters throughout the exercise test to achieve the best possible performance. The achievement of $\text{VO}_{2\text{max}}$ criteria were: 1) oxygen uptake reaching a plateau with increasing work rate; 2) respiratory exchange ratio values >1.1 ; and 3) maximal heart rate (HR_{max}) within 10% of the age-predicted maximal values (21). The maximal aerobic power (watts and watts per kg), maximal aerobic speed (in $\text{km}\cdot\text{h}^{-1}$), and HR_{max} were determined at $\text{VO}_{2\text{max}}$.

Training programs. Before the intervention, tests were performed to ensure that the HIIT cycling and running sessions were isoenergetic. The fasting energy expenditure (EE) induced by a cycling HIIT session (10 x 45s at 80-85% of HR_{max} , 90s active recovery) was calculated in five male participants not included in the study using a K5 apparatus (Edition VII, COMPED). Then, within 48 hours, the same five participants performed also a running HIIT session (x repetitions of 45s at 80-85%, 90s active recovery). For each participant, the number of repetitions was determined to achieve the same EE as during cycling. Overall, the number of repetitions chosen was “9”. The mean EE spent for a cycling or running HIIT session was 281 ± 23 kcal.

Each participant took part in one of the two training programs (HIIT-BIKE or HIIT-RUN). Participants performed three exercise sessions per week for 12 weeks (total session number = 36). Each participant had to complete at least 30 sessions to be included in the analysis. Supervised sessions (approximately 30 min/each) were carried out at the Center of Resources, Expertise and Performance in Sports (CREPS), generally on Monday, Wednesday, and Friday morning, to allow a sufficient recovery period. Each training session was supervised by an experienced certified physical activity instructor.

HIIT-BIKE session. After a 10-min warm-up on the bike (WattBike pro Concept2 including a freewheel and a double air and magnetic braking system), participants performed 10 cycles of 45s of cycling at near-maximal intensity followed by 90s active recovery. The power levels to be produced on the bike were individually determined before the session and corresponded to 80-85% of the HR_{max} during the sprint and 40-45% of the HR_{max} during the recovery phase. The session finished with a 5-min recovery period. Each participant's resistance, pedal cadence (50-70 rpm), HR (A300, Polar, Finland; beat/min) and power (watts) were controlled to reach the expected intensity.

HIIT-RUN session. After a 10-min warm-up on the treadmill (Quasar® h/p/cosmos, Germany), participants performed 9 cycles of 45s of running at near-maximal intensity followed by 90s active recovery. The speed levels to be achieved on the treadmill were individually determined before the session and corresponded to 80-85% of the HR_{max} during the active phase and 40-45% of the HR_{max} during the recovery phase. The session finished with a 5-min recovery. The

treadmill gradient was 1% to simulate real conditions. Each participant's heart rate (A300, Polar, Finland; beat/min) and speed (km/h) were controlled to reach the expected intensity.

The improvement of aerobic capacities required personalized adjustments of power or speed. During each session, each participant was supervised by a physical education instructor to reach the expected intensity.

Physical activity and dietary assessments. Participants were asked to maintain their normal levels of physical activity during the 12-week study period. Their usual weekly level of physical activity was determined at baseline and after the 12 weeks of training (22). They were also asked to maintain their normal eating habits for the study period. At baseline and at week 12 of training, each participant filled in a 7-day food-intake diary that was evaluated by a dietician using a nutrition analysis software (Nutrilog®, Marans, France). A telephone helpline was proposed to participants who experienced problems in completing the 7-day food-intake diary.

Microbiota composition analysis by 16S rRNA Illumina-based sequencing. Participants received a plastic tube to collect their stool within 24 h before the intervention start and 24 h after last exercise session, and were instructed to store the stool sample in a plastic bag in their home freezer before handing it over to the designated person within 24 hours. Upon reception, samples were stabilized in RNA Later (Sigma Aldrich, St. Louis, MO) and stored at -80°C until processing. Genomic DNA was extracted using the Maxwell® RSC PureFood GMO and Authentication Kit (Promega, Madison, WI). The 16S rRNA gene was amplified and sequenced using the Illumina MiSeq technology and the Earth Microbiome Project method with some slight

modifications as previously described (7). Briefly, region V4 of the 16S rRNA gene was PCR-amplified from each sample using composite forward and reverse primers designed with the Golay error correcting code and used to tag the PCR products. The sequence of the forward primer (515F) was 5'-*AATGATACGGCGACCACCGAGATCTACACGCTXXXXXXXXXXXXTATGGTAATTGTGTG*YCAGCGCGTAA-3'. The italicized sequence is the 5' Illumina adapter, the 12 X sequence is the Golay barcode, the bold sequence is the primer pad, the italicized and bold sequence is the primer linker, and the underlined sequence is the conserved bacterial primer 515F. The sequence of the reverse primer (806R) was: 5'-*CAAGCAGAAGACGGCATA*CGAGATAGTCAGCCAGCCGGACTACNVGGGTWTCTAAT-3'. The italicized sequence is the 3' reverse complement sequence of the Illumina adapter, the bold sequence is the primer pad, the italicized and bold sequence is the primer linker, and the underlined sequence is the conserved bacterial primer 806R. PCR reactions included the Hot Master PCR mix (Quantabio, Beverly, MA), 0.2 mM of each primer, and 10–100 ng of template. The reaction conditions were: 95°C for 3 min, followed by 30 cycles (95°C, for 45 s, 50°C for 60 s, and 72°C for 90 s) on a BioRad thermocycler. PCR products were quantified with the Quant-iT PicoGreen dsDNA assay. Then, a master DNA pool was generated from the purified products in equimolar ratios and purified with Ampure magnetic purification beads (Agencourt, Brea, CA). The pooled product was quantified using the Quant-iT PicoGreen dsDNA assay and then sequenced using an Illumina MiSeq sequencer (paired-end reads, 2 x 250 bp) at the Cochin Institute Genom'IC sequencing facility, France. The 16S rRNA sequences were analyzed with Quantitative Insights Into Microbial Ecology (QIIME2, Flagstaff, AZCA) version 2019.7. Sequences were demultiplexed and quality-filtered using the Dada2 method with QIIME2

default parameters to detect and correct Illumina amplicon sequence data, and a table of QIIME2 Amplicon Sequence Variants (ASVs) was generated. Then, the α -diversity of bacterial communities was assessed by calculating the Shannon's diversity index, and β -diversity was used to analyze the dissimilarity among the group membership and structure. Unweighted UniFrac distances were reported according to the principal coordinates analysis (PCoA). For taxonomic analysis, ASVs were assigned to operational taxonomic units with a 99% threshold of pairwise identity to the Greengenes reference database 13.8.

Biochemical assays. Blood samples were collected 1 week before the program start (baseline values) and 2-4 days after the last training session. After overnight fasting, a cannula was inserted in an antecubital vein, and blood was collected in EDTA- and fluoride-containing vacutainers tubes. The plasma concentration of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, ultrasensitive reactive C protein, glucose and insulin were immediately measured at an analysis center. The HOMA-IR index was calculated using the formula: $\text{HOMA-IR} = [\text{fasting glucose (mmol}\cdot\text{L}^{-1}) \times \text{fasting insulin (}\mu\text{U}\cdot\text{mL}^{-1})] / 22.5$.

Statistical analyses

All statistical analyses were carried out with the STATISTICA version 12.00 software (StatSoft 266 Inc., Tulsa, OK, USA). Data are presented as the mean \pm standard deviation (SD). The data normal distribution was tested using the Kolmogorov–Smirnov test, and the homogeneity of variance was tested using the F-test. When necessary, data were log-transformed before analysis. Two-way analysis of variance (ANOVA) with repeated measures was used to

determine group and time effects, and group \times time interactions. When a significant effect was found, post-hoc multiple comparisons were performed using the Newman-Keuls test. When significant main or interaction effects were detected, the effect size was assessed using the partial eta-squared (η^2) and ranked as follows: ~ 0.01 = small effect, ~ 0.06 = moderate effect, ≥ 0.14 = large effect (23). Baseline values and changes between baseline and the study end [Δ change: (12 weeks–baseline/baseline) \times 100] were also compared between groups, using the non-parametric Mann–Whitney U test. Spearman correlation was used to determine correlations between body composition, metabolic profile and gut microbiota parameters. Differences with a P-value ≤ 0.05 were considered significant.

RESULTS

Participant's characteristics

Only 20 of the initial 24 participants met the eligibility criteria. These 20 participants were randomly divided into the two exercise groups: HIIT-RUN (n = 10) and HIIT-BIKE (n = 10). One participant had a hamstring injury while using the treadmill and withdrew from the study. There was no other reported adverse event during testing or training in both groups. However, two participants withdrew from the study due to personal reasons, and one participant contracted COVID-19. Therefore, only 16 participants [HIIT-RUN (n = 8), HIIT-BIKE (n = 8)] completed the training program and were included in the statistical analysis (see flowchart in Figure 1).

At baseline, mean age and physical fitness (VO_{2max}) were not different between groups (52.9 \pm 10.3 vs. 55.7 \pm 9.3 years and 30.6 \pm 4.8 vs. 34.9 \pm 8.3 ml.min⁻¹.kg⁻¹ for the HIIT-BIKE and

HIIT-RUN groups, respectively; $p > 0.05$). All 16 participants completed the 36 sessions of training except one who missed two sessions.

Habitual energy intake and physical activity level. The daily energy intake (Kcal) and the levels of physical activity (PAL) did not change during the intervention period in both groups and were not different between groups ($p > 0.05$) (Supplemental Table 1, Supplemental Digital Content, Mean daily energy intake, macronutrient intake, and physical activity level in the HIIT-BIKE and HIIT-RUN groups at baseline and at the end of the 12-week intervention).

Anthropometric measurements and body composition. At baseline, anthropometric measurements and body composition were similar between groups (Table 1). Overall, the 12-week intervention induced a significant decrease of body mass (kg), total FM (kg) and waist circumference (cm) (time effect, $p = 0.005$, $\eta^2 = 0.442$; $p = 0.017$, $\eta^2 = 0.342$; $p = 0.006$, $\eta^2 = 0.422$, respectively) (Table 1). When absolute values were expressed as percentage of body weight (%BW), FFM and total soft tissue (*i.e.* FFM minus bone mineral content) tended to increase during the study period ($p = 0.066$ and $p = 0.079$, respectively, with large effects: $\eta^2 = 0.204$ and $\eta^2 = 0.221$) (Table 1).

Total abdominal and visceral fat mass. At baseline, total abdominal FM (kg) and visceral FM (kg) were similar between groups. Overall, both physical activity programs induced a decrease in total abdominal FM (kg) and visceral FM (kg) (time effect, $p < 0.001$, $\eta^2 = 0.748$; $p = 0.001$, $\eta^2 = 0.546$, respectively) (Table 1). When expressed as Δ change values, total abdominal FM loss was

higher in the HIIT-RUN group (-16.1% vs. -8.3%; $p=0.050$) (Fig. 2). No group effect was noted for the percentage of total FM and visceral FM changes (Fig. 2).

Metabolic profile. The glycemic and lipid parameter values at baseline and after the 12-week intervention are listed in Table 2. Glycaemia and HOMA-IR were quite elevated in both groups at baseline (normal range: glycemia = 4-5.4 mmol.L⁻¹ and HOMA-IR ≤ 2.4) (Table 2), whereas the blood lipid profiles did not show any significant metabolic alteration. Overall, glycemia decreased after the training programs (time effect, $p=0.013$, $\eta^2 = 0.369$), but not insulinemia and HOMA-IR. Total cholesterol, HDL-C, LDL-C and triglycerides were not modified by the intervention.

Fecal microbiota composition

Analysis of the fecal microbiota composition by 16S rRNA sequencing showed similar baseline α -diversities (Shannon's diversity index) between groups (Fig. 3A). Moreover, α -diversity was not influenced by the intervention (Fig. 3A), and the Δ change values of the Shannon's diversity index were only associated with total abdominal FM change ($r = -0.6$, $p = 0.016$) (Fig. 3B and 3C). The PCoA of the unweighted UniFrac distances demonstrated clustering based on individual subjects (Supplemental Fig. 1A, Supplemental Digital Content, PCoA plots of Unifrac distance metrics for both HIIT-BIKE and HIIT-RUN groups). The baseline and post-intervention samples of each participant often clustered closely (Supplemental Fig. 1B, Supplemental Digital Content).

Before and after the intervention, the Bacillota/Bacteroidota ratios (*i.e.* Firmicutes/Bacteroidetes in the former nomenclature) were not different between groups. Similarly, the taxonomic analysis did not reveal any significant group difference at the phylum, order and family levels before and after the 12-week programs (Fig. 4). However, overall, both HIIT programs induced changes in the abundance of several families (Fig. 5), with a significant increase of *Rikenellaceae*, *Clostridiaceae* and *Actinomyocetaceae* (time effect, $p < 0.05$). *Christensenellaceae* abundance tended to increase, but without reaching significance (time effect, $p = 0.07$).

Next, a Spearman correlation analysis was performed to determine correlations between changes in body composition or glycemic and lipid profiles and i) the baseline relative abundance of specific microbiota members (Fig. 6A) and ii) changes in the relative abundance of specific microbiota members (Fig. 6B). Figure 6 shows only significant associations. Total FM change was negatively associated with the baseline *Ruminococcus*, *Erysipelotrichaceae* abundances and positively associated with the baseline *Lachnospira* abundance. Total abdominal FM was positively associated with the baseline abundance of *Desulfovibrionaceae* and *Barnesiellaceae*, whereas visceral FM change was negatively associated with the baseline abundance of *Blautia* and *Ruminococcaceae*. Total FM changes were negatively associated with changes in the relative abundance of *Rikenellaceae*, *Mogibacteriaceae*, *Oscillospira* and *Odoribacter*. Moreover, total abdominal FM changes were negatively correlated with *Oscillospira*, *Coprococcus* and *Ruminococcus* relative changes. Visceral FM changes were positively associated with *Verrucomicrobiaceae*, *Allobaculum*, *Akkermansia* and *Ruminococcus* relative changes. Collectively, these findings indicate the presence of correlations between the

host's response to HIIT programs and changes in the intestinal microbiota. They also highlight the association of specific microbiota members with the potential effectiveness of the HIIT programs.

DISCUSSION

The aim of this study was to compare the effects of two 12-week HIIT isoenergetic programs (cycling *vs* running) on body composition and fecal microbiota in non-dieting men with overweight or obesity. Overall, HIIT programs led to a reduction of body weight, total body FM, total abdominal FM and visceral FM. However, total abdominal FM loss was higher in the running group. The fecal microbiota diversity, measured through the α -diversity, remained stable over time and showed no significant difference between groups. However, α -diversity changes were associated with the percentage of abdominal FM reduction. Additionally, specific microbial families in the baseline fecal microbiota and post-intervention changes were correlated with total body, total abdominal or visceral FM changes.

FM accumulation and its unfavorable distribution in the abdominal area contribute to increase CVD risks (1). Consistent engagement in physical activity can be an effective approach to prevent and counteract age-related increases in whole-body and abdominal FM. According to the current international guidelines, endurance training is generally recommended as the most effective strategy for weight loss and for FM reduction in men and women (24). In agreement with several reviews and meta-analyses (5,8,10), our group demonstrated that HIIT also is a safe and time-efficient strategy to reduce total and (intra)-abdominal FM in men and in pre- and post-menopausal women (6,9).

In the present study, we hypothesized that isoenergetic cycling and running HIIT programs would be effective in decreasing total body and (intra-)abdominal FM deposits and that the running HIIT program would induce larger effects. This hypothesis was based on studies showing that running elicits greater cardiorespiratory responses (O_2 consumption and heart rate) during incremental and submaximal exercise, at matched relative and absolute workloads above and below the anaerobic threshold (25,26). Furthermore, at the same percentage of VO_{2max} or maximum workload, the rate of fat oxidation is higher in running (27) and plasma lactate concentrations are higher in cycling, reflecting a greater carbohydrate utilization (and consequently a lower fat oxidation) (11). Running and cycling HIIT protocols with the same duration result in inherently different workloads and O_2 consumption, leading to different physiological and metabolic responses (28). This is due to the larger muscle mass recruitment during running in association with stretch-shortening cycles, including concentric and eccentric phases (27). To avoid such bias, we normalized the HIIT session types to achieve a consistent energy expenditure. Therefore, the session duration was not the same (~20 min of running and 22.5 min of cycling). This normalization may partly explain the similar total body FM loss observed in both groups after the HIIT programs. Nevertheless, we hypothesized that despite the isoenergetic conditions, the running activity eccentric nature would induce: i) higher EPOC and higher lipid oxidation (as already shown by Cunha et al., 2016 after acute exercises) and ii) a potential increase in resting metabolism rate (RMR), thereby facilitating FM loss. Indeed, besides factors such as exercise intensity and duration, the exercise mode may play a critical role in post-exercise metabolism. Muscle damage is more frequent after eccentric-type than concentric-type exercise, and this effect is amplified at high intensities (29). Due to the high energy cost associated with protein resynthesis (which could contribute to 20% of resting

metabolic rate-RMR), some authors suggest that the increased EE resulting from muscle damage might lead to prolonged elevations not only of EPOC but also of RMR up to 48 h post-exercise (11,29). In our study, total body FM loss was not higher in the running than cycling group after the 12-week training period. The repetition of running sessions may have rapidly curbed muscle damage through adaptation mechanisms, potentially restraining the effects on EPOC and RMR.

On the other hand, our study found a more pronounced reduction in total abdominal FM following the running HIIT program (-16.1 in the running vs -8.3%, in the cycling group). Therefore, for a similar total body FM loss, running HIIT might lead to higher abdominal lipolysis. However, this result was not confirmed when we analyzed specifically the visceral adipose tissue, where only a trend was observed ($p = 0.13$). This may be due to the greater heterogeneity of the participants' values and/or to the fact that exercise mostly activates lipolysis in the subcutaneous adipose tissue. Indeed, only 5–10% of circulating long-chain fatty acids are released from visceral adipose tissue in individuals with normal weight (30). The reasons underlying the higher abdominal lipolytic activity during running HIIT remain speculative. Although formal evidence is lacking, various hypotheses can be mentioned. The most plausible hypothesis is related to differences in blood flow irrigating the abdominal regions. Although this has never been demonstrated, the body positioning in cycling vs. running (bent and upright) might generate hemodynamic effects that alter the "respiratory pump mechanism" (31). Moreover, the "muscle pump" activation from ground contacts (or their absence) influences the venous return (32). These adaptations could collectively generate effects that affect blood drawing into the trunk (31). Thus, for a similar catecholamine production (which remains to be demonstrated at the same relative intensity due to differences in the involved muscle mass) (14),

running HIIT could facilitate exercise-induced lipolysis at the abdominal level, explaining the greater loss of abdominal adipose tissue after the 12 weeks of training.

Our study did not find any significant difference in metabolic parameters between groups after the 12-week physical activity program. However, we did observe a time effect concerning fasting plasma glucose, with lower values after the training period. Unfortunately, both HIIT programs did not lead to improvements in other parameters. However, this is not surprising because the baseline lipid profile values were already within the normal ranges in both groups.

Emerging research indicates that exercise promotes a balanced gut microbiota (33–35), and several studies demonstrated that physical activity favors an increase in beneficial microbial species, enhances the gut microbiota diversity, and fosters the growth of commensal bacteria, all of them contributing to various health benefits (34,35). On the other hand, reduced microbiota diversity has been linked to obesity, type 2 diabetes, and impaired blood glucose regulation (15,33).

To the best of our knowledge, our study is the first to assess and compare the effects of running *vs.* cycling HIIT on body FM by taking into account also the potential influence of the gut microbiota composition. This hypothesis was based on the finding that in athletes, gut microbiota composition varies depending on the sport discipline (36), suggesting a potential effect of physical training modalities on the microbiome. In 2015, Allen et al. were the first to demonstrate in mice that voluntary exercise (wheel) and forced exercise (treadmill) differently alter gut microbiota composition (37). Currently, there is strong evidence that the α - and β -

diversity of the gut microbiota are influenced more strongly by high intensity than low intensity exercises. Additionally, in clinical populations, exercising 4 to 5 times per week seems to offer more advantages compared to only 2 or 3 times (38). In our study, we did not observe any significant difference in the α - and β -diversity of the gut microbiota after both programs. In humans, the absence of effect on α -diversity after training interventions is quite common (7,39,40), especially during short- and medium-term physical activity programs (41). Despite this lack of effect, our data highlighted that α -diversity changes were negatively associated with total abdominal FM loss, strengthening the hypothesis of a connection between FM loss and bacterial richness of the gut. Results on the β -diversity are more controversial in the literature. Some authors did not detect any β -diversity difference in lean and obese individuals after few months of training (40,42,43), whereas others demonstrated significant changes (39,44). In our study, we did not compare the effect of the two HIIT programs on β -diversity with a control group; however, when comparing the two training groups, we did not identify any cluster. Overall, the lack of difference in α - and β -diversity between groups might be explained by the fact that the training interventions were i) isoenergetic, ii) at the same intensity, and iii) at the same frequency with nearly identical durations. More studies are necessary to conclude whether running and cycling HIIT programs of longer duration might have different effects on gut microbiota composition.

Physical training may also modulate the relative abundance of specific phyla, families, and bacterial species (33,42,43). Overall, our data showed that the two HIIT programs induced similar changes in the abundance of different families. Notably, we observed a significant increase in *Rikenellaceae* and *Clostridiaceae*, with a trend concerning *Christensenellaceae*

($p=0.07$). The abundance of these three families is reduced in individuals with obesity compared to lean individuals (45). These findings also corroborate our previous laboratory study showing an increase in *Christensenellaceae* after treadmill HIIT training in rodents (16). Furthermore, a study suggested that humans with a gut microbiota enriched in *Christensenellaceae* and *Rikenellaceae* display lower levels of visceral adipose tissue (46). Here, we found that higher abundance of *Rikenellaceae* and *Oscillospira* was negatively associated with greater FM loss. Additionally, *Oscillospira* variation was strongly correlated with changes in abdominal FM. Notably, *Oscillospira*, which is less abundant in individuals with high BMI, appears to have health-promoting properties, particularly in the context of obesity (47). Finally, FM loss was negatively correlated with *Odoribacter* abundance. Recently, it has been demonstrated that *Odoribacter laneus* improves glucose tolerance and reduces inflammatory markers in rodent models of obesity, making it a promising probiotic candidate (48). We also investigated potential correlations between baseline microbiota composition and changes in body composition and metabolic profiles induced by the training program. Overall, we observed a negative correlation between the relative abundance of *Blautia* at baseline and visceral FM loss, suggesting that higher baseline levels of *Blautia* were associated with greater reductions in visceral FM. Interestingly, some studies indicated that *Blautia* abundance is higher in individuals with lower visceral fat, and an increase in *Blautia* has been linked to reduction in visceral FM (49). A recent study also suggested that *Blautia wexlerae* may have beneficial effects in obesity by modulating lipid metabolism and reducing inflammation (50).

Collectively, these correlations between bacterial families and body composition highlight two points: i) the initial gut microbiota composition may influence HIIT-induced body

composition changes, and ii) modulating the abundance of specific bacteria might influence HIIT-induced body composition changes, as we previously observed in menopausal women (7).

One of the limitations of this study is the small number of participants and the absence of a control group without physical activity. Although our sample size was sufficient to demonstrate, as expected, a significant loss of (intra)-abdominal FM after HIIT training, the high interindividual variability in fecal microbiota composition made it challenging to compare the two exercise modalities. Another limitation was the absence of continuous diet monitoring throughout the study period. Diet was only recorded using a 7-day food-intake diary at baseline and at week 12. We cannot ensure that the diet remained stable between these time points and/or that specific components were not introduced, potentially influencing the modulation of gut microbiota composition (51).

CONCLUSIONS

Both cycling and running isoenergetic HIIT programs improved the body composition of individuals with overweight or obesity, suggesting that the training program can be adapted to the participant's preferences and/or capacities. The intestinal microbiota composition at the study start and its post-intervention changes were correlated with FM reduction, highlighting the potential connection between these factors. However, additional studies are required to better decipher the mechanisms underlying the greater loss of abdominal FM observed in the running HIIT group.

Acknowledgments

The authors thank the Genom'IC platform (INSERM U1016, Paris, France) for help. The results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

Funding

This work was supported by the “Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation (MESRI), Clermont Auvergne University (AME2P laboratory), Inserm (Institut national de la santé et de la recherche médicale; UMR1071), INRAE (Institut national de recherche en agriculture, alimentation et environnement; USC 1382), the Agence Nationale de la Recherche of the French government through the program “Investissements d'Avenir” (16-IDEX-0001 I-SITE project CAP 20-25 project) of Clermont Auvergne University. BC's laboratory is supported by a Starting Grant from the European Research Council (ERC) under the European Union Horizon 2020 research and innovation program (grant agreement No. ERC-2018-StG- 804135), an award from the Fondation de l'avenir (AP-RM-21-032), the ANR grants EMULBIONT (ANR-21-CE15-0042-01) and DREAM (ANR-20-PAMR-0002) and the national program “Microbiote” from INSERM.

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FIGURE LEGENDS

Figure 1: Flowchart of the participants' recruitment.

HIIT-RUN: Running high-intensity interval training; HIIT-BIKE: cycling high-intensity interval training

Figure 2: Fat mass changes between baseline and the end of the 12-week training program in the HIIT-BIKE (n = 8) and HIIT-RUN (n =8) groups. Data are the mean \pm SD. HIIT: high-intensity interval training; FM: fat mass; Delta change (%) = [(12 weeks – baseline/baseline) \times 100]. *: p \leq 0.05: HIIT-BIKE vs. HIIT-RUN.

Figure 3: Alpha-diversity at baseline (pre) and at the study end (post) in the HIIT-BIKE (n=8) and HIIT-RUN (n=8) groups (A). Correlations between Shannon's index changes and body composition and blood metabolic parameter changes (B). Correlation between Shannon's index changes and total abdominal FM changes (C). Δ : delta change (%) = [(12 weeks – baseline/baseline) \times 100]. T, time; G, group; G x T, group x time interaction.

Figure 4: Relative abundance of bacterial families in the fecal microbiota before (Pre) and after (Post) the 12-week program in the HIIT-BIKE (n=8) and HIIT-RUN (n=8) groups.

Figure 5: Changes in the relative abundance of four gut bacterial families between baseline (Pre) and the end of the 12-week training program (Post) in the HIIT-BIKE (n = 8) and HIIT-RUN (n =8) groups. T, time; G, group; G x T, group x time interaction.

Figure 6: Associations between changes in body composition or glycemic and lipid profiles and baseline relative abundance of specific bacteria (A) and changes in the relative abundance of specific bacteria (B).

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SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplemental Digital Content.docx

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Figure 1

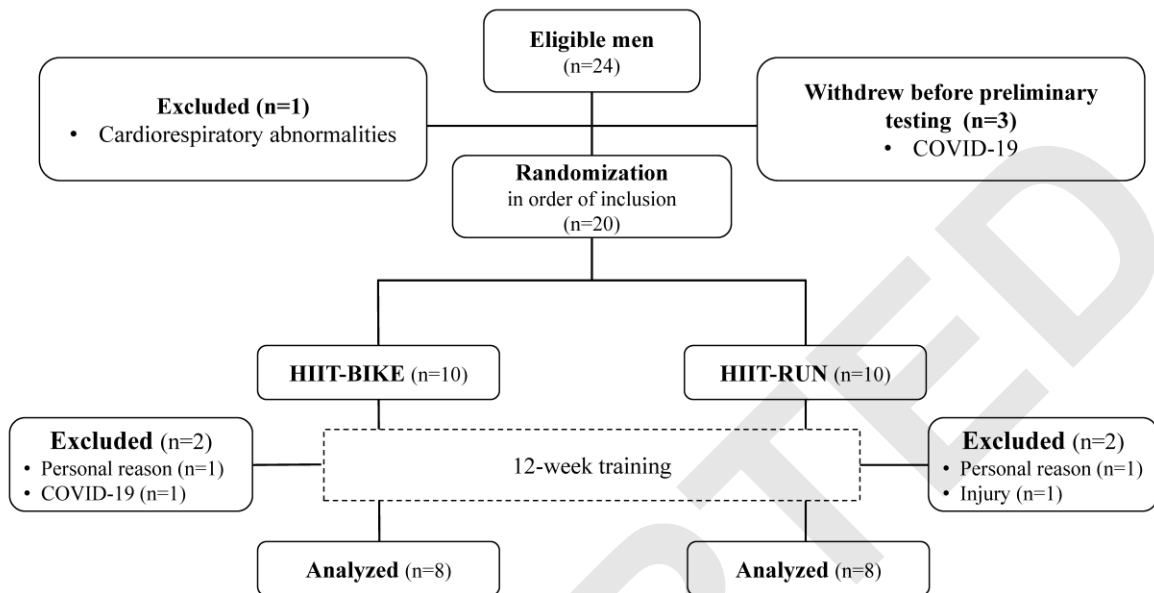
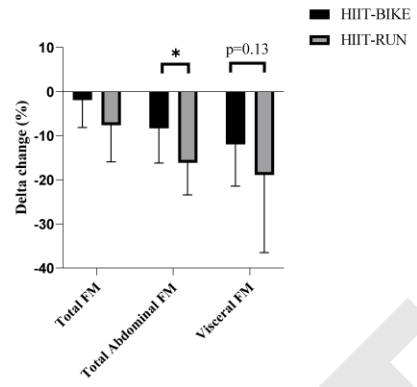


Figure 2



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Figure 3

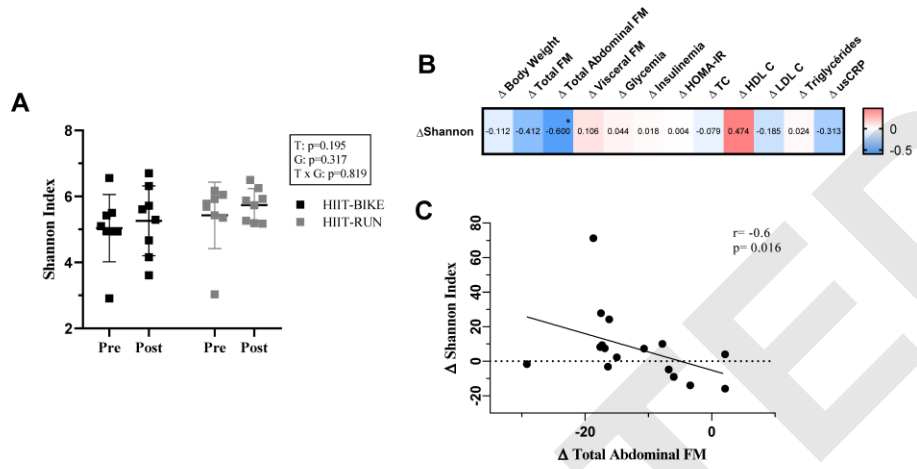


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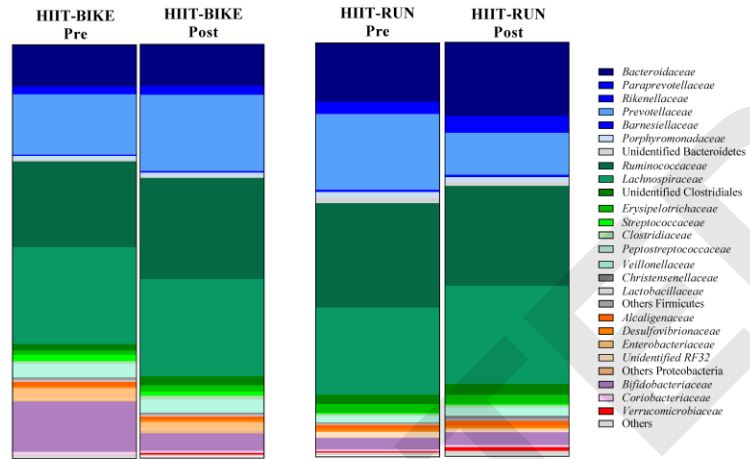


Figure 5

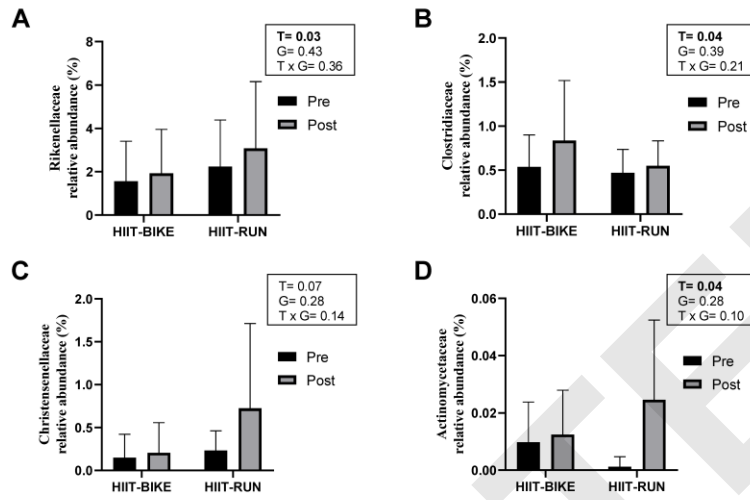


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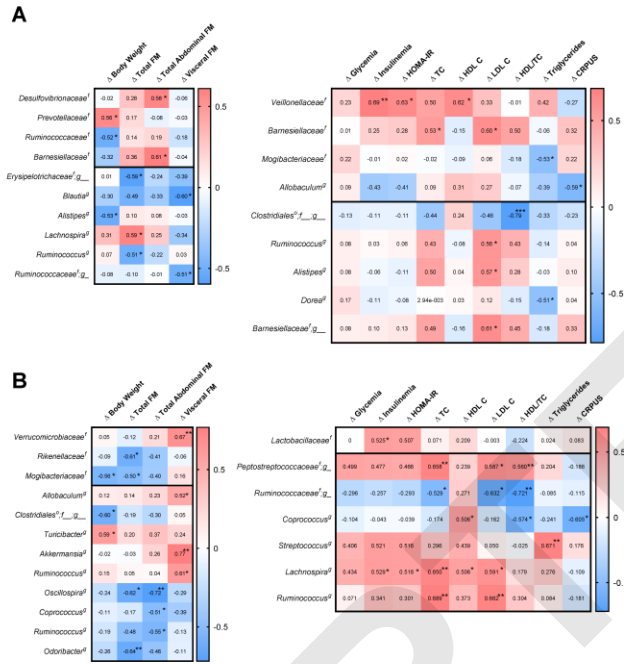


Table 1: Body composition and physical fitness in the HIIT-BIKE and HIIT-RUN groups at baseline (pre) and at the end (post) of the 12-week intervention.

	HIIT-BIKE		HIIT-RUN		ANOVA (p), η^2		
	Pre	Post	Pre	Post	G	T	G x T
BODY COMPOSITION							
BMI (kg.m ⁻²)	30.7 ± 2.8	30.4 ± 3.0	29.2 ± 1.5	28.6 ± 1.6	0.175	0.005	0.293
					0.127	0.448	0.078
Body Mass (kg)	89.5 ± 7.0	88.7 ± 7.9	90.9 ± 7.2	89.1 ± 6.5	0.803	0.005	0.224
					0.005	0.442	0.104
WC (cm)	106.7 ± 6.5	104.4 ± 7.5	100.9 ± 6.5	100.0 ± 8.1	0.175	0.006	0.198
					0.127	0.422	0.198
HC (cm)	101.2 ± 4.1	100.7 ± 5.8	101.1 ± 4.5	100.0 ± 5.1	0.879	0.183	0.605
					0.002	0.123	0.020
Total FM (kg)	21.7 ± 4.1	21.3 ± 4.4	19.0 ± 4.7	17.7 ± 4.9	0.178	0.017	0.155
					0.125	0.342	0.139
Total FM (%)	24.2 ± 3.1	23.9 ± 3.5	20.8 ± 4.2	19.7 ± 4.6	0.067	0.078	0.221
					0.220	0.204	0.105
Total FFM (kg)	67.7 ± 4.4	67.3 ± 5.4	71.7 ± 5.2	71.4 ± 4.8	0.114	0.365	0.977
					0.168	0.059	<0.001
Total FFM (%)	75.8 ± 3.1	76.0 ± 3.5	79.2 ± 4.2	80.3 ± 4.5	0.063	0.066	0.227
					0.226	0.221	0.102
Total ST Mass (kg)	65.2 ± 4.3	64.9 ± 5.4	68.9 ± 5.0	68.6 ± 4.6	0.142	0.389	0.987
					0.147	0.053	<0.001
Total ST Mass (%)	73.0 ± 2.7	73.2 ± 2.1	76.0 ± 4.0	77.1 ± 4.3	0.071	0.079	0.237
					0.214	0.204	0.098
Total Abdominal FM (kg)	6.2 ± 1.2	5.7 ± 1.2	5.3 ± 1.3	4.5 ± 1.3	0.105	<0.001	0.186
					0.177	0.748	0.121
Visceral FM (kg)	2.8 ± 0.5	2.4 ± 0.6	2.3 ± 0.4	1.8 ± 0.5	0.039	0.001	0.506
					0.269	0.546	0.032

Values are presented as mean ± SD.

Soft Tissue Mass (ST) = FFM – bone mineral content by dual-energy x-ray absorptiometry.

G, group effect; T, time effect; G x T, group–time interaction; BM, body mass. Bold significant differences between pre- and post-intervention values, significant P values (≤ 0.05) and eta-squared ($\eta^2 \geq 0.14$ (i.e., large effect)).

Table 2: Metabolic parameters in the HIIT-BIKE and HIIT-RUN groups at baseline (pre) and after (post) the 12-week intervention

	HIIT-BIKE		HIIT-RUN		ANOVA (p), η^2		
	Pre	Post	Pre	Post	G	T	G x T
Glycemia (mmol.L ⁻¹)	5.85 ± 0.86	5.47 ± 0.70	5.34 ± 0.33	5.25 ± 0.43	0.237	0.013	0.097
					0.098	0.369	0.184
Insulinemia (μU.mL ⁻¹)	13.61 ± 5.02	12.00 ± 2.75	6.26 ± 1.40	6.10 ± 1.90	< 0.001	0.425	0.510
					0.685	0.049	0.034
HOMA-IR	3.57 ± 1.42	2.93 ± 0.79	1.49 ± 0.36	1.41 ± 0.45	< 0.001	0.255	0.366
					0.679	0.098	0.063
TC (mmol.L ⁻¹)	5.19 ± 1.03	5.22 ± 0.90	5.46 ± 1.44	5.28 ± 0.74	0.750	0.689	0.566
					0.007	0.012	0.024
HDL-Cholesterol (mmol.L ⁻¹)	1.34 ± 0.18	1.41 ± 0.35	1.36 ± 0.27	1.41 ± 0.28	0.948	0.366	0.914
					<0.001	0.059	<0.001
LDL Cholesterol (mmol.L ⁻¹)	3.22 ± 0.93	3.27 ± 0.80	3.61 ± 1.26	3.39 ± 0.59	0.568	0.624	0.419
					0.024	0.018	0.047
TC/HDL-C	3.88 ± 0.69	3.85 ± 0.93	4.05 ± 0.84	3.81 ± 0.58	0.843	0.461	0.557
					0.003	0.039	0.025
TG (mmol.L ⁻¹)	1.39 ± 0.47	1.48 ± 0.40	1.10 ± 0.22	1.05 ± 0.22	0.045	0.718	0.228
					0.257	0.010	0.102
usCRP (mg.L ⁻¹)	2.51 ± 1.78	3.20 ± 2.64	1.75 ± 1.50	1.95 ± 1.77	0.297	0.191	0.463
					0.077	0.119	0.039

Values are presented as mean ± SD.

G, group effect; T, time effect; G x T, group–time interaction.

TG, triglycerides; usCRP, ultrasensitive C reactive protein.

Bold significant eta-squared (η^2) ≥ 0.14 (i.e., large effect).

	HIIT-BIKE		HIIT-RUN		ANOVA (p), η^2		
	Pre	Post	Pre	Post	G	T	G x T
Energy intake (Kcal)	2160 ± 247	2134 ± 394	2240 ± 514	2143 ± 327	0.80	0.56	0.74
Proteins (g)	79.5 ± 13.1	85.8 ± 7.1	99.4 ± 25.3	89.7 ± 13.0	0.11	0.73	0.13
Fat (g)	94.9 ± 19.3	87.4 ± 23.1	84.1 ± 29.7	91.8 ± 16.6	0.76	0.99	0.22
CHO (g)	219.1 ± 33.0	221.3 ± 30.9	227.4 ± 48.0	192.4 ± 52.7	0.59	0.20	0.15
Physical Activity Level (PAL)	1.67 ± 0.11	1.65 ± 0.12	1.62 ± 0.15	1.72 ± 0.28	0.96	0.39	0.19

Supplemental Table 1: Mean daily energy intake (Kcal), macronutrient intakes (g) and physical activity level (PAL, defined as the total daily energy expenditure to daily basal metabolism ratio) in the HIIT-BIKE and HIIT-RUN groups at baseline (pre) and at the end (post) of the 12-week intervention. CHO: carbohydrates. Values are the mean ± SD. G, group effect; T, time effect; G x T, group–time interaction.

Supplemental Figure 1: Principal Coordinates Analysis (PCoA) plots of Unifrac distance metrics for both HIIT-BIKE (n=8) and HIIT-RUN (n=8) groups. In **A**, dots are colored by patient ID, and the arrows present microbiota evolution from pre-intervention to post-intervention. In **B**, dots are colored by experimental group and timepoint.

