HIIT discontinuation may be detrimental to diabesity parameters whereas retraining enhances intestinal health and gut microbiota diversity

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Research Article

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Abstract

Background

The term "diabesity," which refers to the simultaneous presence of diabetes and obesity, is associated with a significant mortality rate globally. However, there is a lack of comprehensive literature on the effects of stopping high-intensity interval training (HIIT) and then resuming it. This study sought to examine how the interruption and subsequent resumption of HIIT impacted the physiological changes in mice with diabesity. Seventy-five C57BL6 mice were used in this study, divided into five stages: baseline, diabesity induction, training, detraining, and retraining. Diabesity was induced in two groups of mice ( obese - Western Diet, and control - diet AIN) over 10 weeks. Both groups were further divided into control and trained animals, with each stage (training, detraining, and retraining) lasting 6 weeks. HIIT was performed three times a week, consisting of five sets at 90% of maximum speed ($V_{\text{max}}$). This study also analyzed various parameters, including lipid profile, glycemic kinetics, blood glucose, adiposity index, intestinal histology, and gut microbiota profile.

Results

HIIT resulted in metabolic enhancements and a fat loss, whereas the detraining led to a decline in these alterations. Although retraining exhibits a degree of blood glucose regulation, it was not effective in generating fat loss and weight loss. Conversely, HIIT retraining seems to enhance goblet cell populations and increase gut crypt depth, concurrently causing modifications in the gut microbiota composition. While both the initial training and subsequent retraining exerted an effect on the composition of the gut microbiota, the impact of a Western diet demonstrates a more substantial potency compared to that of physical training concerning intestinal health and microbial composition.

Conclusions

These findings may contribute to a broader understanding of diabesity management and introduce novel perspectives for the use of physical training to enhance patient outcomes in gut microbiota composition.

Introduction

The term "diabesity" describes the combined health consequences arising from the coexistence of obesity and type II diabetes[1]. Globally, the obesity incidence increased by a factor of six over a 40-year span reaching an estimated 800 million adults [2]. Simultaneously, type II diabetes has affected approximately 415 million individuals [3]. Consequently, this dual epidemic exerts significant financial implications on a global scale, as evident from the staggering $1.31 trillion attributed to diabetes-related costs in 2015 worldwide [4]. Projections indicate that healthcare expenditures related to obesity-associated conditions are set to reach around $1 trillion in the United States by 2025 [2].
Diabesity pathophysiology involves mechanisms encompassing modifications in beta cell function, adipose tissue biology, and insulin resistance, affecting multiple organs [5]. A sedentary lifestyle and adherence to a Western diet, characterized by high fat and carbohydrate content and low fiber intake, are amongst the contributing factors for diabesity development [6]. Regular exercise enhanced glycemic control, insulin signaling, and blood lipids, improved vascular function, as well as gut microbiota alterations and weight loss [7, 8]. Among the various forms of exercise recommended for managing diabesity, high-intensity interval training (HIIT) may offer supplementary advantages in terms of improving cardiorespiratory fitness, promoting fat loss and glycemic control [9, 10].

Despite the numerous HIIT benefits in relation to diabesity, there is considerable debate regarding its adherence and suitability for sedentary individuals [11, 12]. Cessation of exercise, or detraining, appears to augment adipose tissue's energy storage capacity [13]. Given that individuals with diabetes type II typically exhibit an average adherence rate of 58% to physical exercise, it is of utmost significance to understand HIIT-stopping action and subsequent retraining. Among these discussions about training and detraining, the concept of retraining emerges as a pivotal yet less-explored facet warranting profound consideration [14]. Additionally, while the impacts of training and detraining on diabesity are being elucidated, the distinct influence of retraining remains unknown, in our understanding. In this regard, the intricate dynamics of retraining, especially in the HIIT context, present an intriguing opportunity to bridge existing gaps and unearth new insights. Moreover, understanding HIIT retraining effects is essential, given the fluctuations in exercise adherence and the potential implications for diabesity management. This context can reveal intricate responses, leading to reversibility of diabesity-associated changes and exercise efficacy. These perspectives guide optimal exercise regimens and interventions for diabesity, enriching strategies and novel perspectives for patient outcomes. This study provides novel insights into the realm of retraining, enhancing our understanding of diabesity management.

**Methods**

**Animals**

The Committee of the Animal Experimentation Center of the Catholic University of Brasilia approved all animal protocols (nº 004/19). The present study was composed of 75 C57BL6 male mice at 4 weeks of age, and the experimental design is presented in the 1st Supplementary Material. After the previous collection (baseline, BL group), 35 animals were submitted to diabesity induction by Western Diet (WD_I group) [15] and another control diet (35 animals) known as AIN diet (A_I group)[16] for 10 weeks. The animals were randomly distributed, and the present study followed the ARRIVE guidelines. From the post-diabesity induction period, they were subdivided into four distinct groups. Two of these groups were trained (AT_T and WT_T), and the other two groups were inactive (AC_T and WC_T), with n = 15 in each group. Three periods were composed of 6 weeks including training, detraining (AC_D, AT_D, WC_D, and WT_D groups) and retraining (AC_R, AT_R, WC_R and WT_R groups). In each period (baseline, obesity induction, training, detraining, and retraining), 5 animals per group were euthanized for biological analysis. The entire experiment lasted for 28 weeks, without the animals reaching old age. The animals
were placed individually in cages to avoid “the cage effect” and to avoid similarities in the microbiota. Water and food were provided *ad libitum*, and the animals were kept on a 12:12 h light-dark cycle in a room at 23 ± 2°C.

**Adaptation and physical training**

All mice were familiarized before randomization on the mice treadmill (Treadmill: Exer 3/6, Columbus Instruments) with 10 m.min⁻¹, 0% incline, with 15 min of duration for 2 weeks. In the 4th week from the beginning of training, that is, after adaptation, an incremental test was applied to detect the aerobic power of the animals. At the beginning of each period (training, detraining, and retraining) all animals performed an incremental maximum velocity test to detect aerobic power. At the end of the maximum speed incremental test, the maximum distance reached by each animal was calculated. The training period (AT_T and WT_T groups) consisted of 6 weeks, 3 days/week, 5 sessions/ day, 60s each session at high intensity, adapted from Denou, Marcinko et al [17]. The applied intensity was 90% of the maximum velocity ($V_{\text{max}}$) that the animals reached in the maximum velocity test. The active rest period between the 5 sets was 1 minute with 50% of the $V_{\text{max}}$. After 6 weeks of training, the trained groups remained for 6 weeks of detraining (AT_D and WT_D groups). Finally, the same training protocol was induced to retrain the trained groups (WT_R and WT_R groups), as presented in the 1st Supplementary Material.

**Glucose tolerance test**

Glucose tolerance test (GTT) was performed according to the guidelines for metabolic tolerance testing in mice [18]. A sterile solution of glucose at 10% (w/v) in phosphate-buffered saline (PBS) was used. Blood glucose was measured after 6 h of fasting. Blood glucose measurements were repeated at 15, 30, 60, 90 and 120 min [19]. The GTT was performed in all periods at the same time: start of fasting at 8:00 am and start of the test at 2:00 pm. The area under the curve of the results obtained in each period was executed.

**Measurements and tissue collection**

The animals' body weight and weekly intake (in grams) were checked by an analytical balance (Shimadzu, AUY220) throughout the experiment. The adiposity index (AI%) was measured by the sum of the weight of retroperitoneal, subcutaneous, epididymal and omental white adipose tissue / body weight x 100 [20]. The caloric value of weekly intake was analyzed by the amount of food placed in its box and the remaining food [21]. At the beginning of each period (baseline, diabesity induction, training, detraining, and retraining), tissues were collected for *in vitro* analyses. Fecal materials were collected 24 h after the last exercise session bout for gut microbiota *in vitro* analysis. Furthermore, feces were collected and analyzed from the same animals that performed all the steps for comparing the gut microbiota between periods and groups. Adipose tissue sites were collected to measure the AI%. Blood was collected by cardiac pulse and soon after centrifuged at 4,000 g for 10 min to separate serum from plasma. The small and large intestines were collected in 10% formalin for histological analysis, which will be described below.

**Extraction of intestines and histological analysis**
Five animals per group were used for histological analysis in all periods of the experiment. First, 2 cm of duodenum was collected for analysis of the depth of the crypts and 2 cm of sigmoid colon for quantification of goblet cells [22]. The pieces were fixed in paraffin. The slides were prepared from 4 µm sagittal sections made with a microtome and stained with hematoxylin-eosin. Image capture was performed with the LAS EZ (Leica Microsystems®). Each measurement was performed on one villus per field with a 10x objective and preferably on a villus that did not show artifacts. To obtain the depth of the crypts, a straight line was drawn between the base of the crypts, that is, at the basal end of the intestinal glands, to the upper portion of the crypts. The count of the number of goblet cells per field was performed in three different fields with a 40x objective. Broken or immature goblet cells were avoided.

**Lipid profile**

Total cholesterol, triglycerides, LDL, and HDL levels were quantified using the Cobas c 111 analyzer and reagents provided by the manufacturer (F. Hoffmann-La Roche Ltd) [23]. The lipid profile was analyzed during all periods of the study, including baseline, post-dietary induction, training, detraining, and retraining.

**Microbial DNA extraction and 16S rRNA gene sequencing**

Total DNA was extracted from feces using the PowerFecal DNA Isolation Kit (MoBio, Carlsbad, CA, USA) according to the manufacturer's instructions. DNA concentrations were determined by spectrophotometry using a Nanodrop (Thermo Scientific). Microbial composition was assessed by 16S rRNA gene metagenomic analysis, performed on an Illumina MiSeq instrument using a v3 Reagent Kit. The libraries followed the Illumina protocol with the primers: 5’- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG − 3’ (V3F) and 5’- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAC − 3’ (V4R). Fragments of the 16S rRNA genes were amplified using polymerase chain reaction (PCR). The amplification process followed a specific order: microbial DNA at a concentration of 5 ng·µL⁻¹, amplicon PCR Primer at a concentration of 1 µM, amplicon PCR Reverse Primer at a concentration of 1 µM, and 2× KAPA HiFi HotStart ReadyMix, with a final reaction volume of 25 µL. The PCR reaction consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 60 s, and extension at 72°C for 90 s. This was followed by a final extension step at 72°C for 7 min and cooling to 10°C. After the PCR amplification, the resulting products were quantified using the Qubit® system (Life Technologies). Subsequently, the amplicons were prepared by attaching barcodes using the Nextera® XT Index Kit. Finally, the prepared samples were run on the MiSeq Illumina platform following the standard protocol (16S Metagenomic Sequencing Library Preparation).

**16S rRNA gene data analysis**

The Illumina adapters located at the 3’ end of the reads were removed using Trim Galore (v. 0.6.4), which can be found at this GitHub repository: https://github.com/FelixKrueger/TrimGalore. The microbiome bioinformatic analysis was conducted using QIIME2 (v.2021.8) [24]. The raw sequence data underwent quality filtering and denoising with DADA2 through the q2-dada2 plugin [25]. To establish a phylogeny, SEPP (Spatially Explicit Phylogenetic Placement) implemented in the q2-fragment-insertion plugin of
Qiime2 was employed [26]. All amplicon sequence variants (ASVs) were aligned against the SILVA-138-99 database [27] using the feature-classifier classify-sklearn method, which employed a Naïve Bayes classifier [28] trained on the Illumina primers specific to the V3-V4 region of the 16S rRNA gene. Subsequent analyses were performed using the MicrobiomeAnalyst web platform available at https://www.microbiomeanalyst.ca/ [29]. Data were filtered using a minimum count threshold of 5 and a low count filter based on 20% prevalence in the samples. Prior to statistical comparisons, the data were rarefied to the minimum library size. Rarefaction curves were generated to examine the relationship between the number of ASVs and the number of sequences. Alpha diversity indices, including observed species, Chao1, Simpson, and Shannon, were assessed. The results were presented through box plots, displaying the diversity indices across samples for each group or experimental period. Differences in diversity indices among sample groups were identified using ANOVA, and the Duncan test was employed as a post-hoc test. Beta diversity was calculated using weighted UniFrac distance metrics and visualized using Principal Coordinates Analysis (PCoA). PERMANOVA (Permutational Multivariate Analysis of Variance) was used to test the strength and statistical significance of sample groupings based on both unweighted UniFrac distances. To ensure the assumptions of PERMANOVA were met, betadisper, a multivariate analogue of Levene's test, was used to verify that differences between groups in terms of their centroids were not due to differences in variances. The differential abundance analysis was performed using the EdgeR method.

**Statistical analysis**

The data obtained from the study underwent comprehensive statistical analysis to explore the underlying patterns and relationships. Principal Component Analysis (PCA) was performed to investigate the structure and variation present in the dataset. This analysis involved identifying the principal components and assessing their contributions to the overall variance observed in the data. The results were visualized using scatter plots, where different groups were represented by distinct colors [30]. PrefMap was employed to highlight variable preferences regarding histological variables (goblet cells and crypt depth). This analysis aimed to provide insights into the relationships between these variables and their impact on the overall structure observed in the dataset. A correlation plot was generated to examine the relationships between the studied variables. This analysis aimed to uncover any significant associations or dependencies between the variables of interest. Moreover, the importance of each variable for principal components PC1 and PC2 was determined. This analysis aimed to identify the variables that made the most significant contributions to the observed patterns and variations in the data [31].

**Results**

**Effects of western diet on diabesity, intestinal morphology and gut microbiota in mice**

After a period of 10 weeks on a Western diet induction (WD_I group), the mice exhibited an increase in glycemic kinetics (Fig. 1A), adiposity index (Fig. 1F), blood glucose (Fig. 1G), and lipid profile (Fig. 1A).
When representing the increments in these variables as percentages, the WD_I group exhibited a 37.9% rise in glycemic kinetics, a 130.4% increase in the adiposity index, and a 17.1% elevation in blood glucose levels. The WD_I group also showed higher caloric intake with a 37.9% increase and stronger correlations with glycemic kinetics, lipid profile and AI% (Fig. 1A, C, D). These findings indicate that dietary induction can lead to diabesity development in the mice. In order to enhance the clarity of the in vivo findings, we have consolidated and presented them in the 2nd Supplementary Material. In addition to promoting diabesity, a diet high in fat and sugar content and low in fiber also caused intestinal morphological changes in the animals, see Fig. 1A, D and the 3rd Supplementary Material. The 3rd and 4th Supplementary Materials contain visual representations of histological data. The Western diet was the main variable responsible for alterations in the β-diversity and α-diversity of the gut microbiota, as shown in Fig. 3A, B, D.

Animals that were fed the control diet (A_I group) exhibited an increase in crypt depth (3rd SM), that was perceived in the PCA analyses (Fig. 1A), as well lower caloric intake (Fig. A, C), and a stronger correlation with goblet cells (Fig. 1D). When examined from a percentage standpoint, it was observed that A_I group displayed a more substantial increase in goblet cells (86.7%) compared to crypt depth (7.5%). Additionally, we observed that the A_I group displayed a distinct microbial diversity (Fig. 3A) and abundance (Fig. 3C). Finally, when examining the Prefmaps-PLS between crypt depth and goblet cells, it appeared that all variables tended to negatively correlate with both analyses, except for triglycerides (Fig. 1B). These findings collectively emphasize the significant impact of dietary intervention on diabesity etiology.

**HIIT showed effects on cardiorespiratory fitness, fat loss, and influenced microbial compositions**

After the six weeks of HIIT, the trained groups (AT_T and WT_T groups) had an increase in $V_{\text{max}}$ and maximum distance (Fig. 2A, B), indicating improvements in cardiorespiratory fitness. Physical training also led to reductions in the adiposity index (Fig. 2G), blood glucose levels (Fig. 2H), and glycemic kinetics (Fig. 2A, B). The WT_T group had caloric intake control compared to the WC_T group, which partly explains the decrease in AI% in this period, see Fig. 2A, B. Except for triglycerides, the lipid profile was more correlated with the Western diet groups (WC_T and WT_T, Fig. 2A, B). In comparison to its control group (WT_T vs WC_T), HIIT reduced adiposity index by 21.5%, caloric intake by 24.5%, glycemic kinetics by 18.6%, blood glucose by 7% and increased by 13.1% at $V_{\text{max}}$.

Regarding the goblet cells and depth of the crypts, the goblet cells obtained a greater correlation with the AT_T group and the depth of the crypts more with the WT_T group (Fig. 2A, B). As mentioned, the dietary intervention seems to have a greater effect on the abundance of the microbiota (Fig. 3C). However, when dietary variation was removed, periods were also shown to influence microbial abundance, see Fig. 3B, D. Furthermore, the HIIT resulted in an increase in *Turicibacter* and *Lachnospiraceae UCG 006* abundance in AT_T group (Fig. 4A), whereas the WT_T group exhibited an increase in *UBA1819* and *Eubacterium*
Xylanophilum abundance compared to their respective control groups (Fig. 4B). The taxonomic modifications concerning phyla and genera are documented within the 5th Supplementary Material.

HIIT detraining impacts on metabolic parameters, cardiorespiratory fitness, and fat gain

Almost all physiological parameters were worse with detraining, as can be seen in Fig. 2C, D, G, H. The most surprising result was the increase in AI% (47%) in the WT_D detraining period, see Fig. 2C, D, G. The adaptations generated by HIIT in blood glucose (Fig. 2H) and glycemic kinetics (Fig. 2C) were almost lost after detraining. The cardiorespiratory fitness ($V_{\text{max}}$ and maximum distance) of the trained groups was like the AC_D group, having lost the adaptation generated by HIIT (no % difference between WT_D and WC_D). Detraining also decreased the number of goblet cells and the dept of crypts (Fig. 2C, D).

Conversely, during the detraining period, the gut microbiota diversity of the WT_D group was higher than that of its control group (WC_D), as shown in Fig. 3B. Furthermore, some genera known to produce butyrate, such as Clostridia vadinBB60 (AT_D group), and Desulfovibrio (WT_D group), showed increased levels during this period (Fig. 4A, B).

Retraining seems incapable of producing the same beneficial effects observed with HIIT

Retraining was performed following the same HIIT protocol. However, retraining was unable to generate a significant AI% and body weight decreases (Fig. 2E, F, G). The marginal percentage shift resulting from retraining, comparing WT_R to WC_R, amounted to only 2.2% in terms of caloric intake, 4.4% in body weight, and 9.4% in adiposity index. Although blood glucose (Fig. 2H) decreased in WT_R compared to WC_R, glycemic kinetics correlated with both these groups compared to control diet groups (Fig. 2E, F). Even the cardiorespiratory fitness of the trained groups (AT_R and WT_R) was not superior to the group that received the control diet and remained sedentary (AC_R), see Fig. E, F. These results indicate for the first time that HIIT retraining was incapable of controlling diabesity. However, the process of retraining demonstrated significant enhancements in the condition of the intestines, as observed through an increase in the number of goblet cells and the depth of crypts, as depicted in Fig. 2E, F, and 3rd, 4th SM. In addition, groups that retrained showed greater gut microbiota diversity and abundance, see Fig. 3B, C, E and 5th SM. There was also a greater abundance of the genera Lachnospiraceae NK4A136 and Clostridia vadinBB60 in the control diet group (AT_R group) and the genera Eubacterium xylanophilum and Desulfovibrio in the WT_R group, see Fig. 4A, B. Thus, although retraining was unable to prevent diabesity, gut morphology and gut microbiota modifications were clearly detected.

Discussion

The current study delves into the impact of discontinuing and subsequently reinitiating HIIT within the diabesity framework. HIIT has emerged as a potential approach for managing blood sugar levels and achieving weight loss [32, 33]. Following the diabesity induction in mice through a Western diet, we
surprisingly uncovered a novel finding: halting HIIT leads to the resurgence of diabesity, and retraining did not counteract these effects.

The rationales underlying the potential efficacy of HIIT as a promising strategy for addressing diabesity encompass the use of muscle glycogen, heightened insulin sensitivity, enhanced adipose tissue reduction, elevated cardiorespiratory fitness, modulation of gut microbiota composition, augmented caloric expenditure, and regulation of appetite [34–36]. In the current investigation, we observed outcomes from HIIT that align with those previously documented in existing literature (depicted in Fig. 2A, B, G, H). From a historical vantage point, given that HIIT yields such favorable outcomes and demands less time for execution, it has engendered a transformative shift in global physical activity engagement [37]. Nevertheless, within the realm of diabesity management, the cessation and subsequent reintroduction of HIIT remain uncharted territory, devoid of documented evidence.

The literature is notably limited in its exploration of the effects associated with the detraining and subsequent retraining of HIIT in mice afflicted with diabesity. Among humans, a period of 4 weeks dedicated to HIIT detraining (following an 8-week training regimen) has demonstrated superiority over moderate training in terms of positively impacting the cardiometabolic health of individuals who are overweight [38]. Intriguingly, a span of 4 weeks for HIIT detraining (after a 12-week training interval) undertaken within the context of cyclic normobaric hypoxia was also capable of reducing trunk adiposity in women classified as overweight or obese [39]. Individuals with impaired glucose tolerance and/or a body mass index > 27 kg.m$^{-2}$ maintained the VO$_2$$_{max}$ and HOMA-IR after 3-weeks of HIIT detraining (after 6 weeks of training) [40]. However, our data provides evidence that the HIIT detraining led to heightened adiposity index, augmented caloric intake, amplified glycemic kinetics (as indicated by GTT.AUC), and diminished performance in incremental testing, as depicted in Fig. 2C, D, G. Here, the application times of HIIT training and detraining were also equalized. Furthermore, the parameters encompassing intensities, active rest intervals, overall training duration, and weekly session frequency differed from those employed in the aforementioned studies.

Studies investigating HIIT retraining are even scarcer than detraining. To date, only one study has explored HIIT retraining, focusing on its impact on reducing blood pressure in hypertensive adults [41]. This study revealed a decrease in blood glucose levels resulting from HIIT retraining, a finding consistent with our own observations as depicted in Fig. 2E, F, H. However, since there are no studies of HIIT retraining on diabesity, the present study is pioneering in revealing the constraints of HIIT retraining concerning obesity and glycemic kinetics. Thus, despite the use of HIIT for addressing diabesity [42], our main findings suggest that implementing it with an intervening hiatus may not yield an effective strategy for achieving fat loss.

Parallel to the previously presented data, the consequences of HIIT detraining and subsequent retraining on gut health and the composition of gut microbiota remained unexplored. Thus, we also investigated the effects of HIIT detraining and retraining on intestinal health and gut microbiota on diabesity, shown in Fig. 3, 4. The gut microbiota has been approached as one of the central factors in the diabesity
pathophysiology of [43]. Imbalances in gut microbial composition, referred to as dysbiosis, have the potential to contribute to various pathological conditions, diabesity included [44]. In this context, the substantial impact of diabesity induction on gut microbiota abundance is vividly illustrated in Fig. 3C. Additionally, it has already been discussed in the literature that 6 weeks of HIIT alters the gut microbiota composition [17]. In this sense, our investigation discerned that both HIIT and subsequent retraining could induce modifications in the alpha- and beta-diversity, as well as in certain genera within the gut microbiota, as evident in Figs. 3 and 4. Butyrate-producing genera such as *Eubacterium xylanophilum* and *Desulfovibrio* genus (and its phylum *Desulfobacterota*) were increased through HIIT retraining in animals with diabesity. Furthermore, HIIT and retraining exhibited the capacity to enhance intestinal health by fostering augmented associations with goblet cells and an increased crypt depth, as depicted in Fig. 2A, B, E, F. Despite the absence of an equivalent impact of HIIT retraining as compared to initial training on factors such as fat reduction and other physiological parameters (as summarized in Fig. 2E, F, G), it becomes apparent that retraining elicits alterations in both intestinal and metabolic domains that offer valuable insights for diabesity management.

**Clinical Implications**

In summary, our findings present a novel insight indicating that stopping HIIT training and returning to it might not be a prudent approach for addressing diabesity. HIIT retraining appears inadequate in promoting fat loss, regulating caloric intake, and managing glycemic kinetics. Conversely, animals afflicted with diabesity that underwent retraining exhibited lowered blood glucose levels, an elevation in goblet cell numbers, and alterations within the gut microbiota composition. While both initial training and retraining displayed an impact on gut microbiota composition, even considering differences in genus, it becomes apparent that the influence of a Western diet holds greater potency than that of physical training. It is important to note that our study does not seek to advocate for or against the use of HIIT. Instead, it reinforces the significance of sustained and continuous physical training as a foundational principle in the therapeutic approach towards individuals with diabesity.

**Limitations**

The primary constraint is that the research was conducted using an animal model (mice) rather than human subjects. The objective of this study was to utilize the animal model to enhance environmental control and facilitate more precise analyses, such as monitoring calorie intake and assessing gut microbiota. Consequently, we exercised caution in drawing conclusions from this study, particularly when extrapolating its findings to the context of diabesity in humans. Therefore, we advocate for future research endeavors that explore the impact of HIIT retraining and other forms of exercise on the management of diabesity in humans.

**Declarations**

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**

Not applicable.

**Availability of data and materials**

Not applicable.

**Competing interests**

The authors declare no competing interests.

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Figures

Figure 1

Multidimensional Exploration post-Western Diet Induction. (a) Principal Component Analysis (PCA) was performed to explore structure and variation in the data. The graph axes represent the main components of the PCA, while the colors indicate different groups in the data. The dispersion of points on the chart reveals distinct patterns, trends, or clusters. (b) PrefMap highlights variable preferences in relation to histological variables (goblet cells and depth crypts). (c) The Correlation Plot shows the correlation between the studied variables. (d) The importance of each variable for the main components PC1 and PC2 is presented. (e) Absolute values of the contribution of each variable to PC1 and PC2. In addition, (f, g) a univariate analysis (Kruskal-Wallis followed by Dunn's post-test) of adiposity index and blood glucose is shown, both *P<0.0001.
Figure 2

A Multivariate Journey through PCA and Importance of Variables in Each Period: Training, Detraining, and Retraining. The figure presents the PCA analysis of data from different study periods: (a) Training, (b) Detraining, and (c) Retraining. The graph axes represent the principal components of PCA, while the colors indicate the different study periods. The dispersion of points in the graph reflects the data distribution in relation to the principal components, revealing distinct patterns, trends, or clusters for each period. Additionally, the figure also shows the importance of each variable for PC1 and PC2 in each study period: (d) training, (e) detraining, and (f) retraining. This multidimensional analysis provides insights into the structure and variations in the data throughout the different study periods.
Figure 3

Alpha and beta-diversity analyses of all samples. **a)** Alpha-diversity measure using Observed, Chao1, Shannon and Simpson index at ASV level across all the samples. The samples are represented on the X-axis and their estimated diversity on the Y-axis. Each sample is colored based on treatment class. **b)** Beta-diversity analysis of control diet (AIN diet) groups using Principal Coordinate Analysis (PCoA) with Bray-Curtis distance and phylogenetic-based weighted UniFrac distances. **c)** Beta-diversity analysis of all groups. **d)** Beta-diversity analysis of Western diet groups.
Both diet treatment and training interventions drive microbial compositions. **a)** Control diet associated with HIIT was able to increase the abundance of species that help host resistance to pathogens, such as *Lachnospiraceae NK4A136* and *Clostridia vadinBB60*, **b)** Retraining associated with the Western diet led to the rise of butyrate-producing genera, such as *Eubacterium xylanophilum* and genus *Desulfovibrio* (and its phylum *Desulfobacterota*). All changes had a difference of $p < 0.05$.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial1Experimentaldesign.png
- Supplementarymaterial2Summaryofinvivoresults.tif
- Supplementarymaterial3Histologyofdepthofcrypts.tif
- Supplementarymaterial4Histologyofgobletcells.tif
- SupplementaryMaterial5Taxonomiccomposition.tif