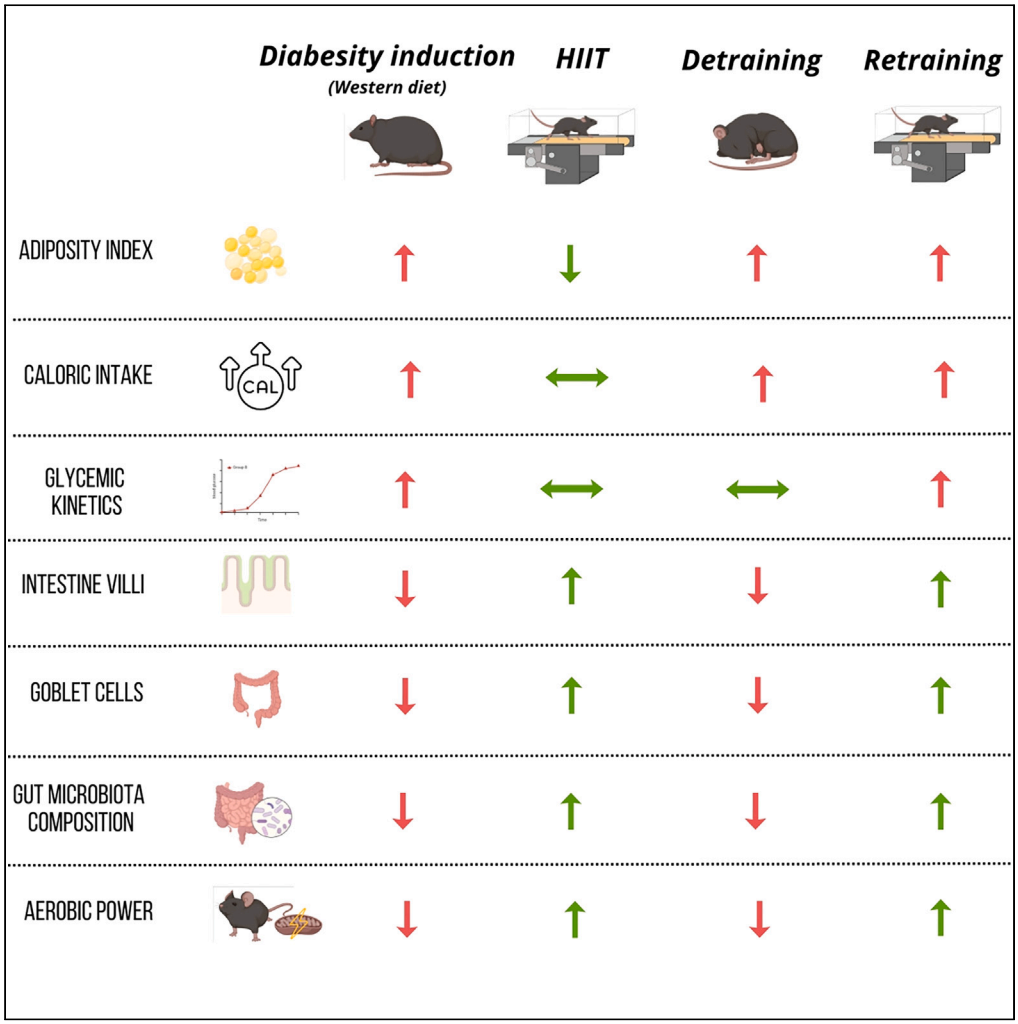
iScience

CellPress
OPEN ACCESS

Article

Discontinuation of HIIT restores diabesity while retraining increases gut microbiota diversity



Filipe Moura Ribeiro, Bernardo Petriz, Maycon Anderson, ..., Osmar Silva, Alinne Castro, Octavio Luiz Franco

ocfranco@gmail.com

Highlights

HIIT detraining led to an increase in body fat and metabolic alterations

Retraining was able to control blood glucose, but not fat loss

Diet has a greater effect on the gut microbiota than HIIT

HIIT and retraining improved gut microbiota

Ribeiro et al., iScience 27, 110365
August 16, 2024
https://doi.org/10.1016/j.isci.2024.110365

iScience

CellPress
OPEN ACCESS

Article

Discontinuation of HIIT restores diabesity while retraining increases gut microbiota diversity

Filipe Moura Ribeiro,[1,2,3] Bernardo Petriz,[1,3,4] Maycon Anderson,[1,3] Victoria Assis,[3,5] Thiago Rosa dos Santos,[1,2] Hugo Correa,[2] Nathalia Cavichiolli de Oliveira,[4] Lana Passos,[1,3] Amanda Fonseca,[1,3] Luiz Arnaldo Brito,[1,3] Osmar Silva,[6] Alinne Castro,[4] and Octavio Luiz Franco[1,2,4,7,*]

SUMMARY

Investigations involving high-intensity interval training (HIIT) have proven to be efficient in controlling diabesity. This study aimed to assess the impact of discontinuing HIIT and retraining within the context of diabesity. 75 C57BL6 mice went through 5 stages: baseline, induction of diabesity with Western diet, training, detraining, and retraining (6 weeks each period). Detraining led to elevated adiposity, exacerbated metabolic parameters and intestinal health, and altered gut microbiota composition. Retraining restored blood glucose regulation and enhanced intestinal health yet did not induce fat reduction. While both training and retraining exerted an effect on the composition of the gut microbiota, the impact of diet demonstrates a more substantial potency compared to that of exercise concerning intestinal health and microbiome. These findings may contribute to a broader understanding of diabesity management and introduce perspectives for the use of specific physical training to enhance patient outcomes and intestine health.

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] In this context, consistent consumption of the Western diet can induce persistent insulin resistance and impair β-cell function.[7] Likewise, physical inactivity can lead to alterations in body composition and dysregulation of insulin receptor proportions in myocytes and adipocytes.[8] Regular exercise enhanced glycemic control, insulin signaling, and blood lipids, improved vascular function, as well as gut microbiota alterations and weight loss.[9,10] 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.[11,12]

Despite the numerous HIIT benefits in relation to diabesity, there is considerable debate regarding its adherence and suitability for sedentary individuals.[13,14] Cessation of exercise, or detraining, appears to augment adipose tissue's energy storage capacity.[15] Given that individuals with diabetes type II typically exhibit an average adherence rate of 53% to physical activity,[16] 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.[17] Additionally, while the impacts of training and detraining on diabesity are being elucidated, the distinct influence of retraining remains unknown, in our understanding. HIIT induces weight loss and metabolic improvements at a faster rate compared to continuous moderate exercise and discontinuing it may potentially elicit more pronounced

[1]Center for Proteomics and Biochemical Analysis, Postgraduate Program in Genomic Sciences and Biotechnology, Catholic University of Brasília, Brasília, DF, Brazil
[2]Postgraduate Program in Physical Education, Catholic University of Brasília, Brasília, DF, Brazil
[3]Laboratory of Molecular Exercise Physiology, University Center, Brasília, DF, Brazil
[4]Postgraduate Program in Biotechnology, S-Inova Biotech, Dom Bosco Catholic University, Campo Grande, MS, Brazil
[5]Laboratory of Molecular Analysis, Postgraduate Program of Sciences and Technology of Health, University of Brasilia, Brasília, DF, Brazil
[6]Postgraduate Program in Pharmaceutical Sciences, Universidade Evangélica de Goiás, UniEVANGÉLICA, Goiás, GO, Brazil
[7]Lead contact
*Correspondence: ocfranco@gmail.com
https://doi.org/10.1016/j.isci.2024.110365

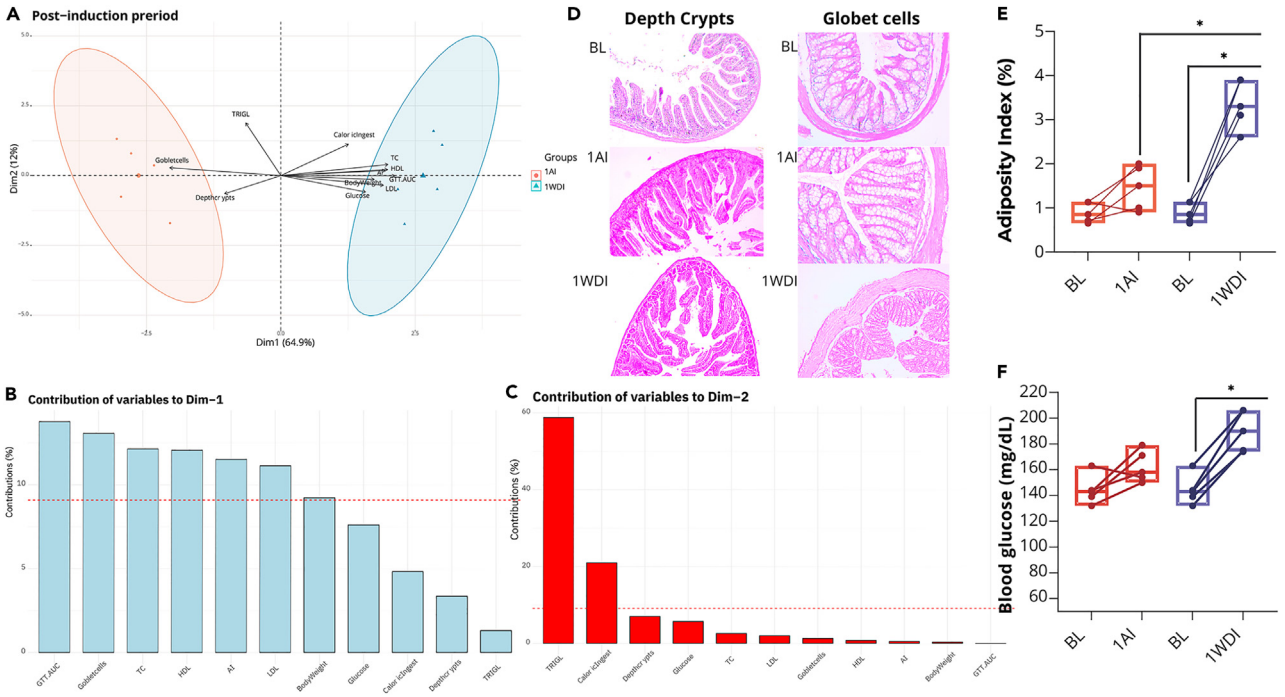


Figure 1. Induction of diabesity through a Western diet consumption

(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 (AIN diet -1AI and Western diet - 1WDI) in the data. The dispersion of points on the chart reveals distinct patterns, trends, or clusters.

(B) The importance of each variable for the main components PC1 and PC2 is presented.

(C) Absolute values of the contribution of each variable to PC1 and PC2.

(D) Histological images from the baseline (beginning of the experiment) and after the AIN diet (1AI) and Western diet (1WDI) interventions.

In addition, (E, F) a univariate analysis (two-way ANOVA followed by Dunn's post-test) of adiposity index and blood glucose is shown, both $*p < 0.0001$.

negative feedback mechanisms in diabesity context.[18] 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.

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 perspectives for patient outcomes. This study offers insights into the cessation of HIIT and its subsequent retraining effects on the physiological parameters of diabesity.

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 (1WDI group), the mice exhibited an increase in glycemic kinetics (symbolized by GTT_AUC in Figure 1A), adiposity index (Figures 1A and 1E), blood glucose (Figures 1A and 1F), TC, LDL, and HDL (Figure 1A). When representing the increments in these variables as percentages, the 1WDI group exhibited a 37.9% rise in glycemic kinetics (GTT_AUC), a 130.4% increase in the adiposity index, and a 17.1% elevation in blood glucose levels. The 1WDI group also showed higher caloric intake with a 37.9% increase and stronger correlations with glycemic kinetics (GTT_AUC), some parameters of lipid profile and AI% (Figure 2A). These findings indicate that dietary induction can lead to diabesity development in the mice. To enhance the clarity of the *in vivo* findings, we have consolidated and presented them in the Figure S3. 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 Figure 1D. The Figure S4 contain visual representations of histological data of AIN groups which are not shown in Figures 1D, 3D, and 3E. The Western diet was the main variable responsible for alterations in the α-diversity and β-diversity of the gut microbiota, as shown in Figures 5A–5C.

Animals that were fed the control diet (1AI group) exhibited an increase in crypt depth (Figures 1D and S3), that was also perceived in the PCA analyses (Figure 1A), as well lower caloric intake (Figure 1A), and a stronger correlation with goblet cells (Figures 1A, 2A, and 2B). 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 1AI group displayed a distinct microbial diversity (Figure 5A) and abundance (Figure 5B) compared with 1WDI group. β-diversity exhibited the most pronounced alterations due to the dietary intervention, as

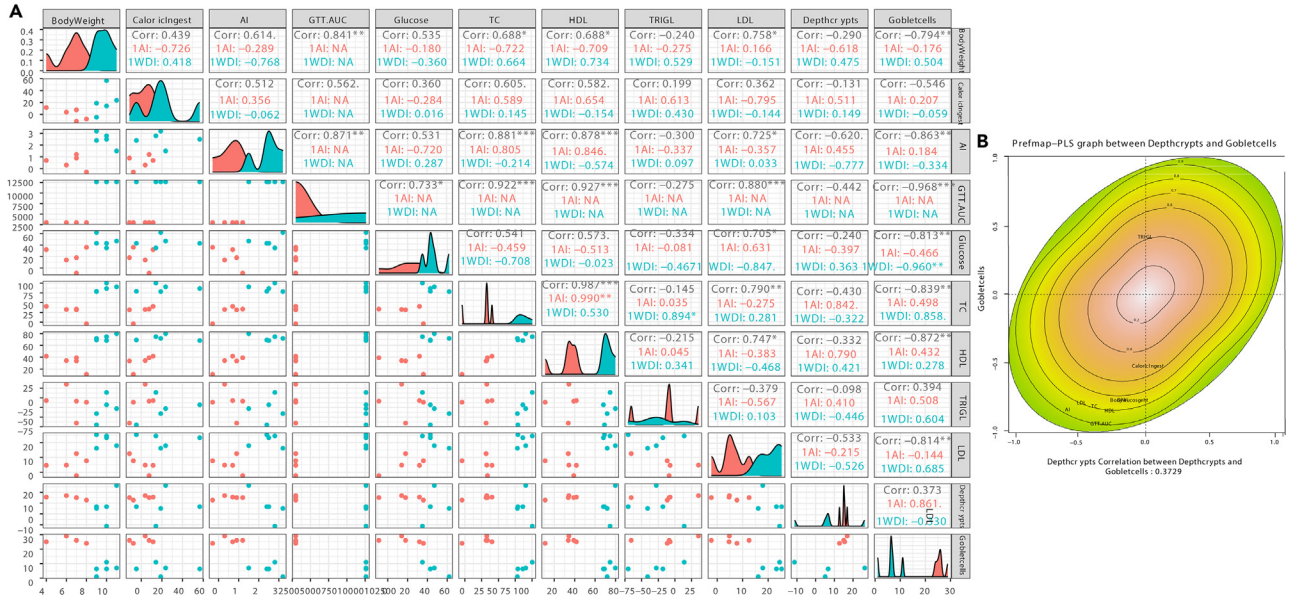


Figure 2. Physiological parameter correlations examined post-dietary intervention

(A) The correlation plot shows the correlation between the studied variables. Statistical differences are indicated by *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

(B) PrefMap highlights variable preferences in relation to histological variables (goblet cells and depth crypts). Baseline: start of experiment (BL); Corr: Correlation; Post-dietary induction: AIN post-dietary induction (1AI); WD post-dietary induction (1WDI); Post-training: AIN control - post-training (2AC); AIN trained post-training (2AT); WD control - post-training (2WC); trained WD - post-training (2WT); Post-detraining: AIN control - post-detraining (3AC); AIN trained post-detraining (3AT); WD control - post-detraining (3WC); trained WD - post-detraining (3WT); Post-retraining: AIN control - post-retraining (4AC); AIN trained post-retraining (4AT); WD control - post-retraining (4WC); WD trained - post-retraining (4WT).

illustrated in Figure 5C. 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 (Figure 2B). These findings collectively emphasize the significant impact of dietary intervention on diabesity etiology.

HIIT showed effects on cardiorespiratory fitness, reduced blood glucose, and influenced microbial compositions

Following six weeks of HIIT, the trained groups (2AT and 2WT) exhibited elevated V_{max} and maximum distance (Figures 3A and 3B) compared to their respective controls (2AC and 2WC groups), indicating enhancements in cardiorespiratory fitness. The 2WT group demonstrated caloric intake regulation compared to the 2WC group, which partly contributed to the observed decline in the AI% during this period, albeit without statistical significance (Figures 3A and 4A). Physical training also resulted in decreases in blood glucose levels (Figures 3A and 4B) and glycemic kinetics (GTT_AUC Figure 3A). Apart from triglycerides, the lipid profile showed stronger correlations with the Western diet groups (2WC and 2WT, Figure 3A). When compared to its control group (2WT vs. 2WC), HIIT reduced caloric intake by 24.5%, glycemic kinetics (GTT_AUC) by 18.6%, blood glucose by 7%, and increased V_{max} by 13.1% (Percentages derived from the comparison depicted in Figure 3A).

Goblet cells exhibited a stronger correlation with the 2AT group, while the depth of the crypts correlated more with the 2WT group (Figures 3D and 3E). As noted, dietary intervention appears to have a more pronounced effect on the abundance and β-diversity of the microbiota (Figures 5B and 5C). However, even when dietary variation was controlled for, time periods were also demonstrated to influence microbial abundance, as depicted in Figures 6A and 6B. Additionally, HIIT led to an increase in *Turicibacter* and *Lachnospiraceae UCG 006* abundance in the 2AT group (Figure 7A), whereas the 2WT group exhibited an increase in *UBA1819* and *Eubacterium xylanophilum* abundance compared to their respective control groups (Figure 7B). Detailed taxonomic modifications regarding phyla and genera are documented in the Figure S5.

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

Almost all physiological parameters were worse at the end of the detraining, as can be seen in Figures 3B, 4A, and 4B. The most surprising result was the increase in AI% (47%) in the 3WT detraining period, see Figure 4A. The adaptations generated by HIIT in glycemic kinetics (GTT_AUC in Figure 3B) and blood glucose (Figures 3B and 4B) were almost lost after detraining. The cardiorespiratory fitness (V_{max} and maximum distance) of the trained groups was like the 3AC group, having lost the adaptation generated by HIIT (no % difference between 3WT and 3WC). At the conclusion of the detraining period, a reduction in the count of goblet cells and the depth of the crypts was likewise noted. (Figures 3D and 3E). Conversely, at the end of the detraining period, the alpha diversity of the gut microbiota in the 3WT group surpassed that of the control group (3WC), as shown in Figure 5B. Furthermore, some genera known to produce butyrate, such as *Clostridia vadinBB60* (3AT group), and *Desulfovibrio* (3WT group), showed increased levels during this period (Figures 4A and 4B).

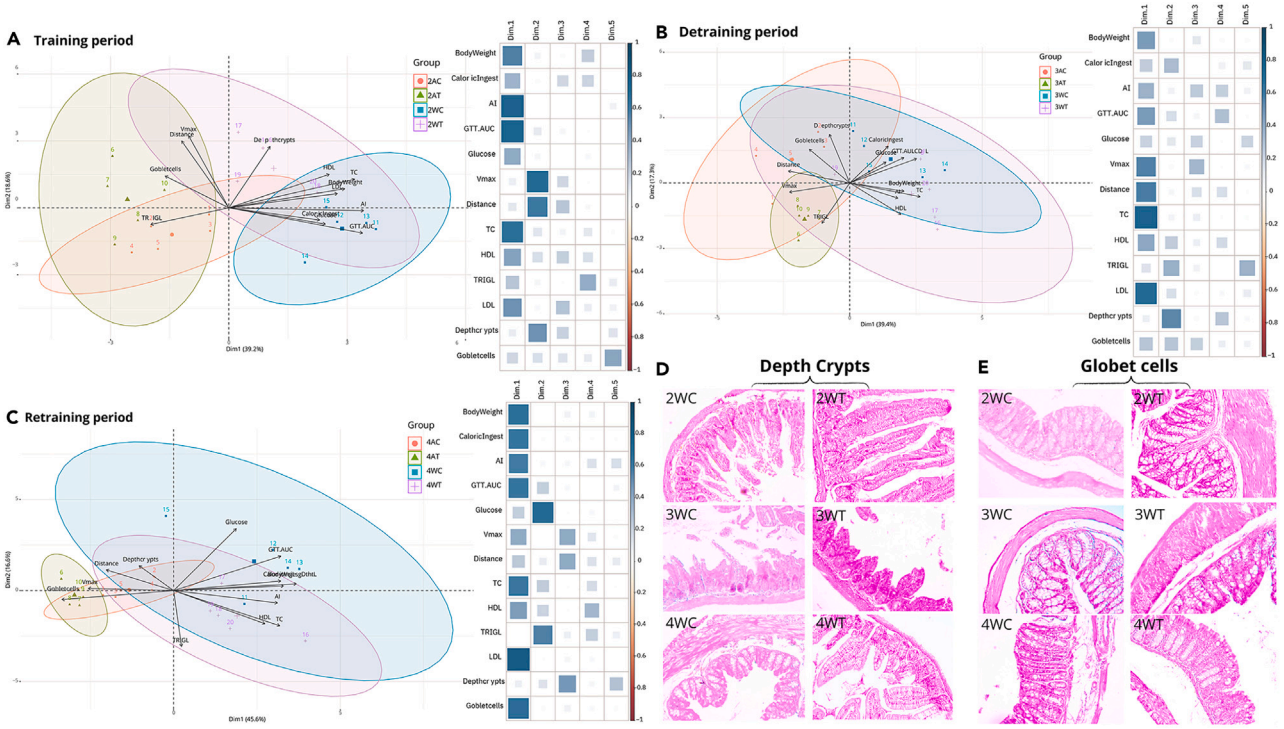


Figure 3. Multivariate analyses of physiological parameters in response to training, detraining, and retraining

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: (A) training, (B) detraining, and (C) retraining. This multidimensional analysis provides insights into the structure and variations in the data throughout the different study periods.

(D) Histological images of the depth of the crypts of the intervention groups (WD control, WC and trained WD, WT) in the three periods: post-training (2WC and 2WT), post-detraining (3WC and 3WT) and post-retraining (4WC and 4WT).

(E) Histological images of the goblet cells of the intervention groups (WC and WT) in the three periods: post-training, post-detraining, and post-retraining.

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 (Figures 2E–2G). 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 (Figure 2H) decreased in WT_R compared to WC_R, glycemic kinetics (GTT_AUC) correlated with both these groups compared to control diet groups (Figures 2E and 2F). 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 findings represent the initial indication that HIIT retraining was ineffective in regulating all parameters associated with 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 Figures 2E, 2F, S3, and S4. Descriptive data on the depth of the crypts and goblet cells were inserted in the Table S1. In addition, groups that retrained showed greater alpha and beta diversity of the gut microbiota, see Figures 3B, 3C, 3E, and S5. 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 *E. xylanophilum* and *Desulfovibrio* in the WT_R group, see Figures 4A and 4B. 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.[19,20] Following the diabesity induction in mice through a Western diet, we surprisingly uncovered an interesting 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

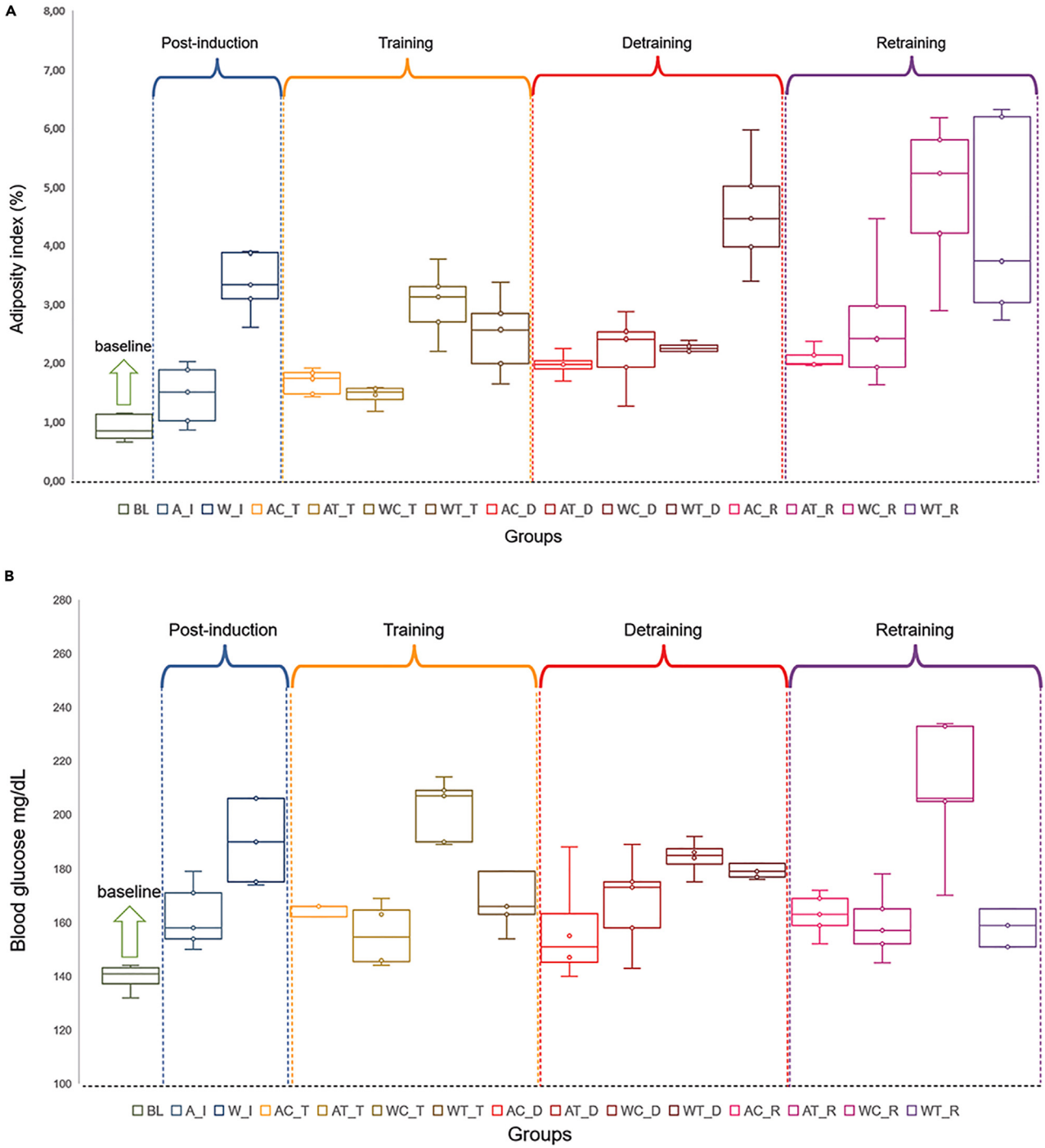


Figure 4. Variations in adiposity index and blood glucose levels over the course of the experiment

(A) The adiposity index and (B) blood glucose are also presented in univariate form. Two-way ANOVA was used to compare dietary induction groups. For comparison between periods, two-way ANOVA with repeated measures followed by posthoc Bonferroni was used. In the analysis of the adiposity index: * 1WDI vs. 1AI ($p < 0.0001$); ** 2WC vs. 2AC and 2AT ($p < 0.01$); # 2WT vs. 3WT and 4WT ($p < 0.01$); *** 3WT vs. all ($p < 0.001$); **** 4WC vs. 4AC and 4AT ($p < 0.009$). In the blood glucose analysis: * 1WDI vs. 1AI ($p < 0.01$); ** 2WC vs. all ($p < 0.01$); *** 4WC vs. all groups ($p < 0.0001$).

composition, augmented caloric expenditure, and regulation of appetite.[21–23] In the current investigation, we observed outcomes from HIIT that align with those previously documented in existing literature (depicted in Figures 3A, 3B, 4A, and 4B). 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

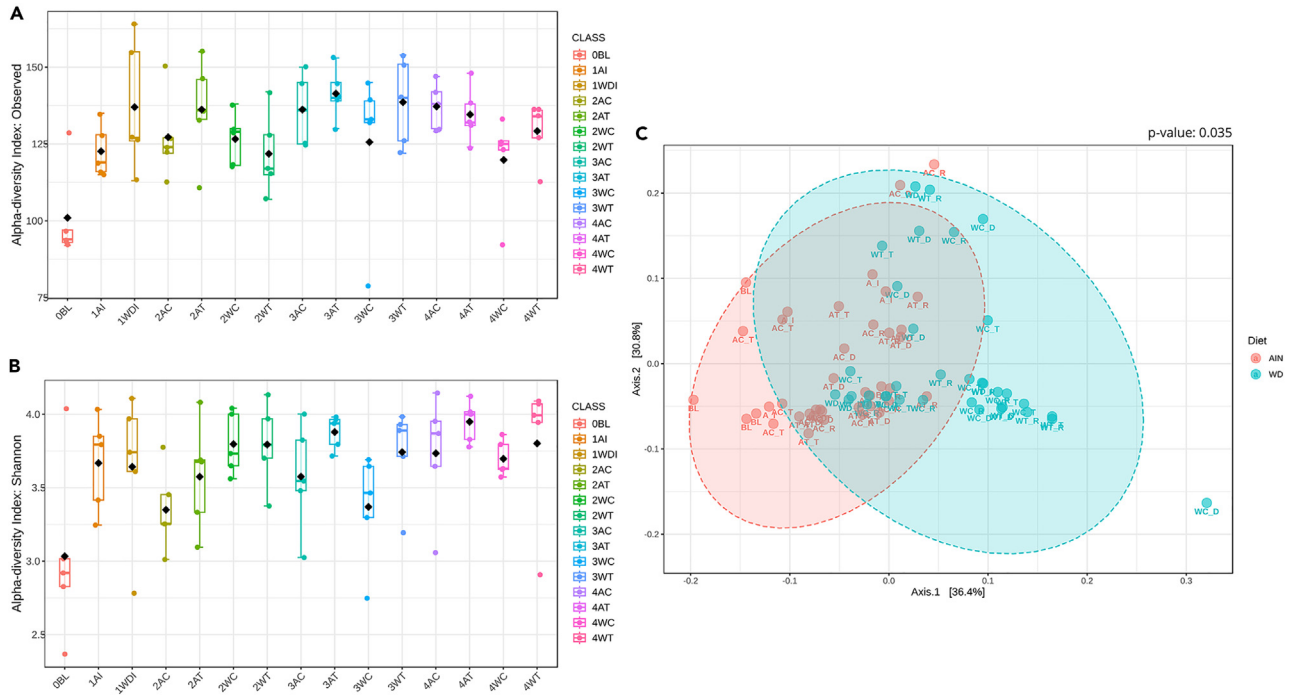


Figure 5. Alpha and beta-diversity analyses

(A) Alpha-diversity measure using Observed 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) Alpha-diversity measure using Shannon index at ASV level across all the samples.

(C) Beta-diversity analysis of all groups using Principal Coordinate Analysis (PCoA) with Bray-Curtis distance and phylogenetic-based weighted UniFrac distances.

physical activity engagement.[24] The impact of physical training on the composition of the microbiome, irrespective of diet, has been extensively deliberated in systematic reviews and even validated in experiments involving mice. Although not as prominently influential on the gut microbiota as diet, the current study revealed that High-Intensity Interval Training (HIIT) induces alterations in microbial composition distinct from those induced by diet (refer to Figures 5B, 6A, and 6B). 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 with overweight.[25] 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 people with obesity.[26] Individuals with impaired glucose tolerance and/or a body mass index >27 kg m^{-2} maintained the $VO2_{max}$ and HOMA-IR after 3-week of HIIT detraining (after 6 weeks of training).[27] 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 Figures 3B and 4A. 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.[28] This study revealed a decrease in blood glucose levels resulting from HIIT retraining, a finding consistent with our own observations as depicted in Figures 3C and 4B. 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,[29] our main findings suggest that implementing it with an intervening hiatus may not yield an effective strategy for achieving fat loss. Hypothesizing the scarcity of literature on the phenomenon of retraining is challenging, and the duration of research could be one contributing factor. The current experimental design offers an opportunity for further investigation into the potential existence of a "gut microbiota memory" regarding the reapplication of various stimuli, extending beyond just physical training.[30,31]

We also investigated the effects of HIIT detraining and retraining on intestinal health and gut microbiota on diabesity, shown in Figures 5, 6, and 7. The gut microbiota has been approached as one of the central factors in the diabesity pathophysiology of.[32] Imbalances in gut microbial composition, referred to as dysbiosis, have the potential to contribute to various pathological conditions, diabesity

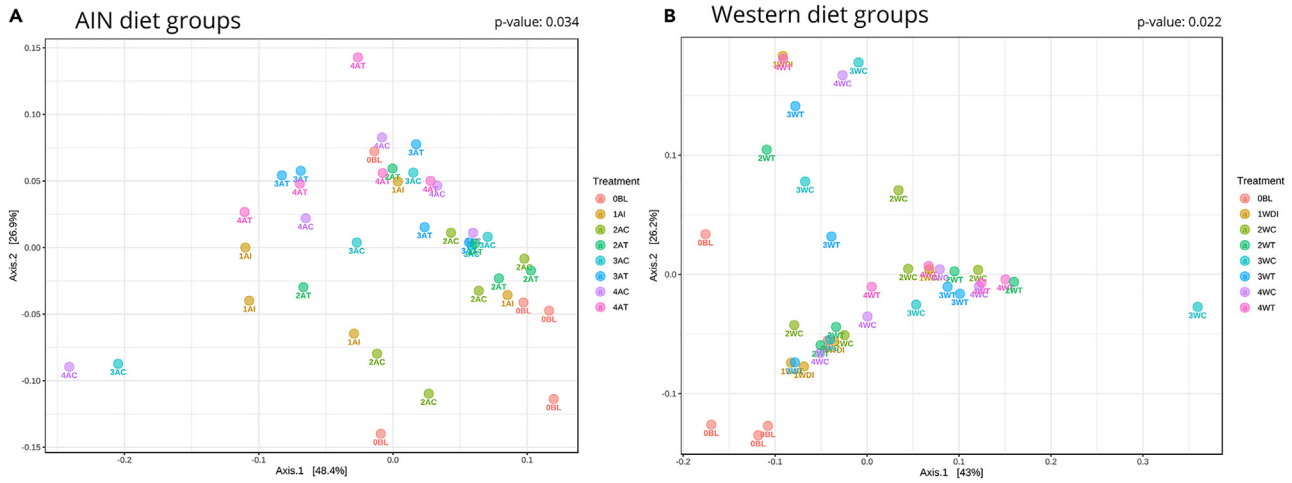


Figure 6. Variations in beta-diversity attributable to the impact of physical training

(A) Beta-diversity analysis of control diet (AIN diet) groups.

(B) Beta-diversity analysis of Western diet groups.

included.[33] In this context, the substantial impact of diabesity induction on gut microbiota abundance is vividly illustrated in Figure 3C. Additionally, it has already been discussed in the literature that 6 weeks of HIIT alters the gut microbiota composition.[34] 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 Figures 5 and 6. Butyrate-producing genera such as *E. xylanophilum* and *Desulfovibrio* genus (and its phylum *Desulfobacterota*) were increased through HIIT retraining in animals with diabesity. These genera are recognized for their positive correlation with glycemic control,[35] which may in part elucidate the observed glycemic control effects of HIIT retraining (refer to Figure 4B). 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 Figures 3A and 3C–3E. 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 Figures 3C, 4A, and 4B), 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 an 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 toward individuals with diabesity.

Limitations of the study

The AIN-93G diet served as the standard diet in this study. It is recognized for its potential to induce metabolic alterations in C57BL6J mice. Despite its lower caloric content, higher fiber content, and distinct macronutrient proportions (refer to Figure S2), the authors advise exercising caution in drawing conclusions regarding the groups fed the AIN-93G diet. 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. 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.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY

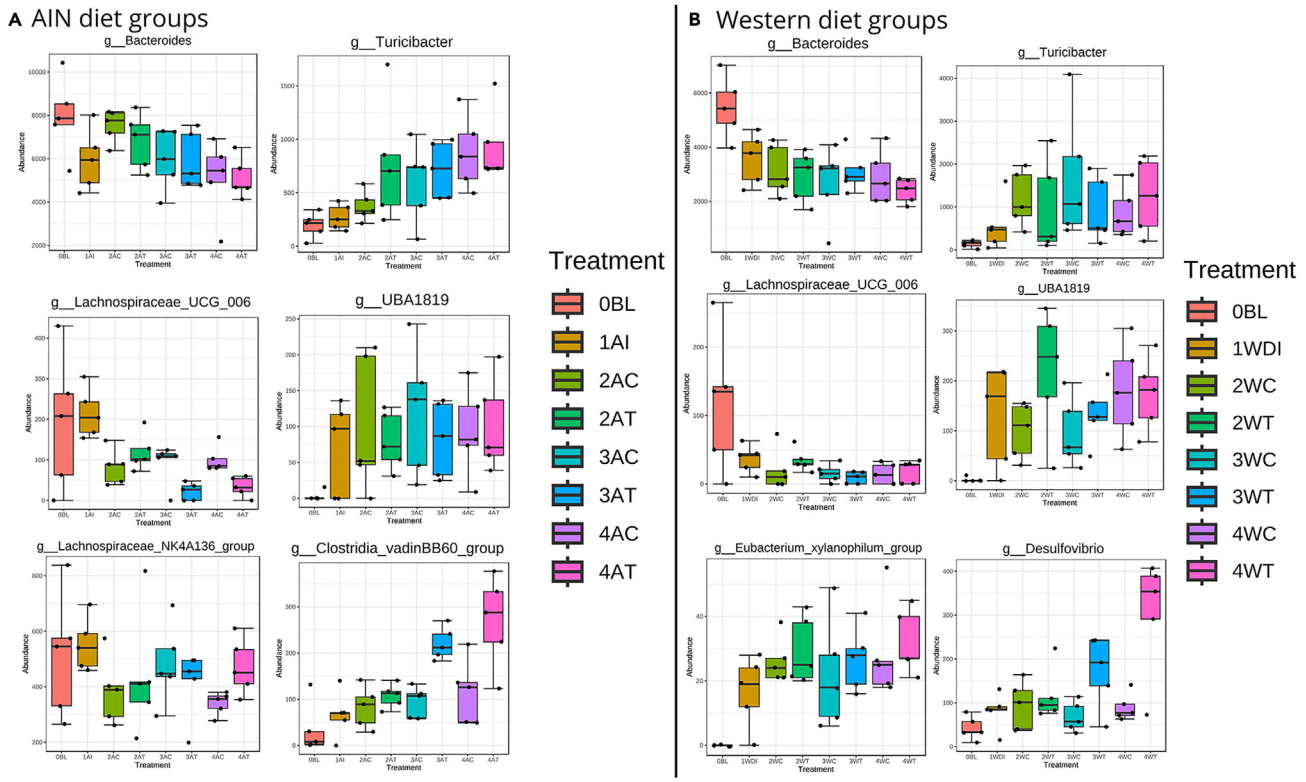


Figure 7. Alterations in microbial composition across each intervention period

(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). Statistical differences are presented in Table S2. All changes had a difference of $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2024.110365.

ACKNOWLEDGMENTS

We would like to thank the Catholic University of Brasília for providing the animals and laboratory facilities necessary to conduct this project. This research was funded by the following Brazilian agencies: the National Council for Scientific and Technological Development (CNPq), the Coordination for the Improvement of Higher Education Personnel (CAPES), the Federal District Research Support Foundation (FAPDF), and the Foundation to Support the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT).

AUTHOR CONTRIBUTIONS

F.R., B.P., and O.F. designed the research. F.R., M.A., V.A., L.P., A.F., and L.A. performed the *in vivo* research; F.R., B.P., T.R., H.C., and O.F. interpreted and analyzed the data; A.C. and N.C. analyzed gut microbiota data; F.R., T.R., and H.C. contributed analytic tools and interpreted the data; F.R. and O.S. contributed to the histological analyses; O.F. supervised and managed the project.

DECLARATION OF INTERESTS

There are no conflicts of interest to disclose.

Received: February 27, 2024
Revised: April 20, 2024
Accepted: June 21, 2024
Published: June 24, 2024

REFERENCES

1. Farag, Y.M.K., and Gaballa, M.R. (2011). Diabesity: an overview of a rising epidemic. Nephrol. Dial. Transplant. *26*, 28–35. https://doi.org/10.1093/ndt/gfq576.
2. Obesity, W. (Day 2022). Available from: https://www.worldobesityday.org/assets/downloads/Obesity_factsheet_one_pager.pdf.
3. Ogurtsova, K., da Rocha Fernandes, J.D., Huang, Y., Linnenkamp, U., Guariguata, L., Cho, N.H., Cavan, D., Shaw, J.E., and Makaroff, L.E. (2017). IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res. Clin. Pract. *128*, 40–50. https://doi.org/10.1016/j.diabres.2017.03.024.
4. Bommer, C., Heesemann, E., Sagalova, V., Manne-Goehler, J., Atun, R., Bärnighausen, T., and Vollmer, S. (2017). The global economic burden of diabetes in adults aged 20-79 years: a cost-of-illness study. Lancet Diabetes Endocrinol. *5*, 423–430. https://doi.org/10.1016/S2213-8587(17)30097-9.
5. Michaelidou, M., Pappachan, J.M., and Jeeyavudeen, M.S. (2023). Management of diabesity: Current concepts. World J. Diabetes *14*, 396–411. https://doi.org/10.4239/wjd.v14.i4.396.
6. Kopp, W. (2019). How Western Diet And Lifestyle Drive The Pandemic Of Obesity And Civilization Diseases. Diabetes Metab. Syndr. Obes. *12*, 2221–2236. https://doi.org/10.2147/DMSO.S216791.
7. Demaria, T.M., Crepaldi, L.D., Costa-Bartuli, E., Branco, J.R., Zancan, P., and Sola-Penna, M. (2023). Once a week consumption of Western diet over twelve weeks promotes sustained insulin resistance and non-alcoholic fat liver disease in C57BL/6 J mice. Sci. Rep. *13*, 3058. https://doi.org/10.1038/s41598-023-30254-2.
8. Eaton, S.B., and Eaton, S.B. (2017). Physical Inactivity, Obesity, and Type 2 Diabetes: An Evolutionary Perspective. Res. Q. Exerc. Sport *88*, 1–8. https://doi.org/10.1080/02701367.2016.1268519.
9. Park, S.S., Kim, T.W., Kim, B.K., Kim, S.H., Park, J.S., and Shin, M.S. (2022). The effects of exercise and diet on mental status, insulin signaling pathway, and microbiome in obese mice. J. Exerc. Rehabil. *18*, 171–178. https://doi.org/10.12965/jer.2244278.139.
10. Ribeiro, F.M., Silva, M.A., Lyssa, V., Marques, G., Lima, H.K., Franco, O.L., and Petriz, B. (2022). The molecular signaling of exercise and obesity in the microbiota-gut-brain axis. Front. Endocrinol. *13*, 927170. https://doi.org/10.3389/fendo.2022.927170.
11. Madsen, S.M., Thorup, A.C., Overgaard, K., and Jeppesen, P.B. (2015). High Intensity Interval Training Improves Glycaemic Control and Pancreatic beta Cell Function of Type 2 Diabetes Patients. PLoS One *10*, e0133286. https://doi.org/10.1371/journal.pone.0133286.
12. Guo, Z., Li, M., Cai, J., Gong, W., Liu, Y., and Liu, Z. (2023). Effect of High-Intensity Interval Training vs. Moderate-Intensity Continuous Training on Fat Loss and Cardiorespiratory Fitness in the Young and Middle-Aged a Systematic Review and Meta-Analysis. Int. J. Environ. Res. Public Health *20*, 4741. https://doi.org/10.3390/ijerph20064741.
13. Santos, A., Lonsdale, C., Lubans, D., Vasconcellos, D., Kapsal, N., Vis-Dunbar, M., and Jung, M.E. (2020). Rates of compliance and adherence to high-intensity interval training in insufficiently active adults: a systematic review and meta-analysis protocol. Syst. Rev. *9*, 56. https://doi.org/10.1186/s13643-020-01301-0.
14. Collado-Mateo, D., Lavin-Perez, A.M., Penacoba, C., Del Coso, J., Leyton-Roman, M., Luque-Casado, A., Gasque, P., Fernandez-Del-Olmo, M.A., and Amado-Alonso, D. (2021). Key Factors Associated with Adherence to Physical Exercise in Patients with Chronic Diseases and Older Adults: An Umbrella Review. Int J Environ Res Public Health *18*, 2023. https://doi.org/10.3390/ijerph18042023.
15. Del Vecchio, F.B., Coswig, V.S., Cabistany, L.D., Orcy, R.B., and Gentil, P. (2020). Effects of exercise cessation on adipose tissue physiological markers related to fat regain: A systematic review. SAGE Open Med. *8*, 2050312120936956. https://doi.org/10.1177/2050312120936956.
16. Jarvie, J.L., Pandey, A., Ayers, C.R., McGavock, J.M., Sénéchal, M., Berry, J.D., Patel, K.V., and McGuire, D.K. (2019). Aerobic Fitness and Adherence to Guideline-Recommended Minimum Physical Activity Among Ambulatory Patients With Type 2 Diabetes Mellitus. Diabetes Care *42*, 1333–1339. https://doi.org/10.2337/dc18-2634.
17. Tokmakidis, S.P., Touvra, A.M., Douda, H.T., Smilios, I., Kotsa, K., and Volaklis, K.A. (2014). Training, detraining, and retraining effects on glycemic control and physical fitness in women with type 2 diabetes. Horm. Metab. Res. *46*, 974–979. https://doi.org/10.1055/s-0034-1390483.
18. Turk, Y., Theel, W., Kasteleyn, M.J., Franssen, F.M.E., Hiemstra, P.S., Rudolphus, A., Taube, C., and Braunstahl, G.J. (2017). High intensity training in obesity: a Meta-analysis. Obes Sci Pract *3*, 258–271. https://doi.org/10.1002/osp4.109.
19. Amri, J., Parastesh, M., Sadegh, M., Latifi, S.A., and Alaee, M. (2019). High-intensity interval training improved fasting blood glucose and lipid profiles in type 2 diabetic rats more than endurance training; possible involvement of irisin and betatrophin. Physiol. Int. *106*, 213–224. https://doi.org/10.1556/2060.106.2019.24.
20. Viana, R.B., Naves, J.P.A., Coswig, V.S., de Lira, C.A.B., Steele, J., Fisher, J.P., and Gentil, P. (2019). Is interval training the magic bullet for fat loss? A systematic review and meta-analysis comparing moderate-intensity continuous training with high-intensity interval training (HIIT). Br. J. Sports Med. *53*, 655–664. https://doi.org/10.1136/bjsports-2018-099928.
21. Chavanelle, V., Boisseau, N., Otero, Y.F., Combaret, L., Dardevet, D., Montaurier, C., Delcros, G., Peltier, S.L., and Sirvent, P. (2017). Effects of high-intensity interval training and moderate-intensity continuous training on glycaemic control and skeletal muscle mitochondrial function in db/db mice. Sci. Rep. *7*, 204. https://doi.org/10.1038/s41598-017-00276-8.
22. de Castro-de-Paiva, P., Marinho, T.d.S., Mandarim-de-Lacerda, C.A., and Aguila, M.B. (2022). Intermittent fasting, high-intensity interval training, or a combination of both have beneficial effects in obese mice with nonalcoholic fatty liver disease. J. Nutr. Biochem. *104*, 108997. https://doi.org/10.1016/j.jnutbio.2022.108997.
23. Shiraev, T., and Barclay, G. (2012). Evidence based exercise - clinical benefits of high intensity interval training. Aust. Fam. Physician *41*, 960–962.
24. Atakan, M.M., Li, Y., Kosar, S.N., Turnagol, H.H., and Yan, X. (2021). Evidence-Based Effects of High-Intensity Interval Training on Exercise Capacity and Health: A Review with Historical Perspective. Int. J Environ. Res. Public Health *18*, 7201. https://doi.org/10.3390/ijerph18137201.
25. Gripp, F., Nava, R.C., Cassilhas, R.C., Esteves, E.A., Magalhães, C.O.D., Dias-Peixoto, M.F., de Castro Magalhães, F., and Amorim, F.T. (2021). HIIT is superior than MICT on cardiometabolic health during training and detraining. Eur. J. Appl. Physiol. *121*,

159–172. https://doi.org/10.1007/s00421-020-04502-6.
26. Camacho-Cardenosa, A., Camacho-Cardenosa, M., Olcina, G., Timón, R., and Brazo-Sayavera, J. (2019). Detraining effect on overweight/obese women after high-intensity interval training in hypoxia. Scand. J. Med. Sci. Sports *29*, 535–543. https://doi.org/10.1111/sms.13380.
27. Phillips, B.E., Kelly, B.M., Lilja, M., Ponce-González, J.G., Brogan, R.J., Morris, D.L., Gustafsson, T., Kraus, W.E., Atherton, P.J., Vollaard, N.B.J., et al. (2017). A Practical and Time-Efficient High-Intensity Interval Training Program Modifies Cardio-Metabolic Risk Factors in Adults with Risk Factors for Type II Diabetes. Front. Endocrinol. *8*, 229. https://doi.org/10.3389/fendo.2017.00229.
28. Avila-Gandia, V., Ramos-Campo, D.J., Garcia-Sanchez, E., Luque-Rubia, A.J., Lopez, A., and Lopez-Roman, F.J. (2023). Training, detraining and retraining effects of moderate vs. high intensity exercise training programme on cardiovascular risk factors. J. Hypertens. *41*, 411–419. https://doi.org/10.1097/HJH.0000000000003346.
29. Guo, Z., Cai, J., Wu, Z., and Gong, W. (2022). Effect of High-Intensity Interval Training Combined with Fasting in the Treatment of Overweight and Obese Adults: A Systematic Review and Meta-Analysis. Int. J. Environ. Res. Public Health *19*, 4638. https://doi.org/10.3390/ijerph19084638.
30. Ribaldone, D.G., Pellicano, R., Fagoonee, S., and Actis, G.C. (2023). Modulation of the gut microbiota: opportunities and regulatory aspects. Minerva Gastroenterol. *69*, 128–140. https://doi.org/10.23736/S2724-5985.22.03152-7.
31. Boccuto, L., Tack, J., Ianiro, G., Abenavoli, L., and Scarpellini, E. (2023). Human Genes Involved in the Interaction between Host and Gut Microbiome: Regulation and Pathogenic Mechanisms. Genes *14*, 857. https://doi.org/10.3390/genes14040857.
32. Ortega, M.A., Fraile-Martinez, O., Naya, I., Garcia-Honduvilla, N., Alvarez-Mon, M., Bujan, J., Asunsolo, A., and de la Torre, B. (2020). Type 2 Diabetes Mellitus Associated with Obesity (Diabesity). The Central Role of Gut Microbiota and Its Translational Applications. Nutrients *12*, 2749. https://doi.org/10.3390/nu12092749.
33. Sharma, A.K., Sharma, A., Lal, S., Kumar, A., Yadav, N.K., Tabassum, F., Sayeed Akhtar, M., Tarique Imam, M., Saeed Almalki, Z., and Mukherjee, M. (2023). Dysbiosis versus diabesity: Pathological signaling and promising therapeutic strategies. Drug Discov. Today *28*, 103558. https://doi.org/10.1016/j.drudis.2023.103558.
34. Denou, E., Marcinko, K., Surette, M.G., Steinberg, G.R., and Schertzer, J.D. (2016). High-intensity exercise training increases the diversity and metabolic capacity of the mouse distal gut microbiota during diet-induced obesity. Am. J. Physiol. Endocrinol. Metab. *310*, E982–E993. https://doi.org/10.1152/ajpendo.00537.2015.
35. Tettamanzi, F., Bagnardi, V., Louca, P., Nogal, A., Monti, G.S., Mambrini, S.P., Lucchetti, E., Maestrini, S., Mazza, S., Rodriguez-Mateos, A., et al. (2021). A High Protein Diet Is More Effective in Improving Insulin Resistance and Glycemic Variability Compared to a Mediterranean Diet-A Cross-Over Controlled Inpatient Dietary Study. Nutrients *13*, 4380. https://doi.org/10.3390/nu13124380.
36. Bortolin, R.C., Vargas, A.R., Gasparotto, J., Chaves, P.R., Schnorr, C.E., Martinello, K.B., Silveira, A.K., Rabelo, T.K., Gelain, D.P., and Moreira, J.C.F. (2018). A new animal diet based on human Western diet is a robust diet-induced obesity model: comparison to high-fat and cafeteria diets in term of metabolic and gut microbiota disruption. Int. J. Obes. *42*, 525–534. https://doi.org/10.1038/ijo.2017.225.
37. Reeves, P.G., Nielsen, F.H., and Fahey, G.C., Jr. (1993). AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. *123*, 1939–1951. https://doi.org/10.1093/jn/123.11.1939.
38. Ferreira, J.C.B., Rolim, N.P.L., Bartholomeu, J.B., Gobatto, C.A., Kokubun, E., and Brum, P.C. (2007). Maximal lactate steady state in running mice: effect of exercise training. Clin. Exp. Pharmacol. Physiol. *34*, 760–765. https://doi.org/10.1111/j.1440-1681.2007.04635.x.
39. Benede-Ubieto, R., Estevez-Vazquez, O., Ramadori, P., Cubero, F.J., and Nevzorova, Y.A. (2020). Guidelines and Considerations for Metabolic Tolerance Tests in Mice. Diabetes Metab Syndr Obes *13*, 439–450. https://doi.org/10.2147/DMSO.S234665.
40. Wang, C.Y., and Liao, J.K. (2012). A mouse model of diet-induced obesity and insulin resistance. Methods Mol. Biol. *821*, 421–433. https://doi.org/10.1007/978-1-61779-430-8_27.
41. Mann, A., Thompson, A., Robbins, N., and Blomkalns, A.L. (2014). Localization, identification, and excision of murine adipose depots. J. Vis. Exp. *94*, 52174. https://doi.org/10.3791/52174.
42. Pugh, T.D., Klopp, R.G., and Weindruch, R. (1999). Controlling caloric consumption: protocols for rodents and rhesus monkeys. Neurobiol. Aging *20*, 157–165. https://doi.org/10.1016/s0197-4580(99)00043-3.
43. Segu, H., Jalsevac, F., Pinent, M., Ardevol, A., Terra, X., and Blay, M.T. (2022). Intestinal Morphometric Changes Induced by a Western-Style Diet in Wistar Rats and GSPE Counter-Regulatory Effect. Nutrients *14*, 2608. https://doi.org/10.3390/nu14132608.
44. Ferreira, C.E.d.S., França, C.N., Correr, C.J., Zucker, M.L., Andriolo, A., and Scartezini, M. (2015). Clinical correlation between a point-of-care testing system and laboratory automation for lipid profile. Clin. Chim. Acta *446*, 263–266. https://doi.org/10.1016/j.cca.2015.04.036.
45. Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. *37*, 852–857. https://doi.org/10.1038/s41587-019-0209-9.
46. Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods *13*, 581–583. https://doi.org/10.1038/nmeth.3869.
47. Mirarab, S., Nguyen, N., and Warnow, T. (2012). SEPP: SATé-enabled phylogenetic placement. Pac. Symp. Biocomput. *2012*, 247–258. https://doi.org/10.1142/9789814366496_0024.
48. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. *41*, D590–D596. https://doi.org/10.1093/nar/gks1219.
49. Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., and Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome *6*, 90. https://doi.org/10.1186/s40168-018-0470-z.
50. Chong, J., Liu, P., Zhou, G., and Xia, J. (2020). Using MicrobiomeAnalyst for comprehensive statistical, functional, and meta-analysis of microbiome data. Nat. Protoc. *15*, 799–821. https://doi.org/10.1038/s41596-019-0264-1.
51. Ihaka, R., and Gentleman, R. (1996). R: a language for data analysis and graphics. J. Comput. Graph Stat. *5*, 299–314. https://doi.org/10.1080/10618600.1996.10474713.
52. Husson, F., Josse, J., Le, S., and Mazet, J. (2012). FactoMineR: multivariate exploratory data analysis and data mining with R. R package version 1.18. https://doi.org/10.18637/jss.v025.i01.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
PowerFecal DNA Isolation Kit	MoBio, Carlsbad, CA, USA	https://www.qiagen.com
v3 Reagent Kit	Illumina MiSeq	www.illumina.com
Nextera® XT Index Kit	Illumina MiSeq	www.illumina.com
Software and algorithms		
R software version 4.3.1	R Core Team	https://www.r-project.org/
QIIME 2 (v.2021.8)	Quantitative Insights Into Microbial Ecology	docs.qiime2.org/2024.2/
Deposited data		
Raw and analyzed data	This paper	SRA data: https://www.ncbi.nlm.nih.gov/sra/PRJNA1121406

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Octavio Luiz Franco (ocfranco@gmail.com).

Materials availability

This study did not generate new reagents.

Data and code availability

- 16s rRNA gene sequences have been deposited at database NCBI and are publicly available as of the date of publication. Accession numbers are listed in the key resources table. The DOI is listed in the key resources table. Microscopy data reported in this paper will be shared by the lead contact upon request.
- All original code has been deposited at NCBI and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The current investigation received prior approval from the Animal Ethics Committee at the Catholic University of Brasília under protocol number 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 Figure S1. C57BL/6 mice were randomly allocated to their respective dietary groups based on body weight and blood glucose measurements, ensuring that there were no significant differences between groups at baseline. After the previous collection (baseline, BL group), 35 animals were submitted to diabesity induction by Western Diet (WD_I group)[36] and another control diet (35 animals) known as AIN diet (A_I group)[37] 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. The entire procedure of anesthetizing the animals was conducted under the supervision and guidance of a veterinarian from the vivarium. Euthanasia was administered through sedation using 2% Xylazine (50 mg kg^{-1}) and 10% Ketamine (80 mg kg^{-1}), followed by cervical dislocation performed by the vivarium veterinarian.

METHODS DETAILS

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. The incremental test commences at a velocity of 13 cm s^{-1}, with increments of 5 cm s^{-1} introduced every 3 min until the subject reaches exhaustion. The final phase represents 100% of the animal's maximal velocity (V_{max}). Termination of the test occurs upon visible exhaustion of the subject, characterized by the inability to sustain the prescribed pace, marked by a duration of 5 s on the shock bar located at the endpoint of the treadmill.[38] Electric shock was administered as a conditioning stimulus for the treadmill and throughout the incremental test. Nevertheless, during the HIIT sessions, manual prodding was employed to mitigate excessive stressors on the animals. 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.[34] The applied intensity was 90% of the V_{max} that the animals reached in the maximum velocity test. The active rest period between the 5 sets was 1 min with 50% of the V_{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 Figure S1. The objective of this study was to apply detraining and retraining protocols in mice while maintaining consistent dietary interventions, with the aim of isolating the effects of training and its variations on diabesity.

Glucose tolerance test

Glucose tolerance test (GTT) was performed according to the guidelines for metabolic tolerance testing in mice.[39] A sterile solution of glucose at 10% (w/v) in phosphate-buffered saline (PBS) was used. The glucose solution was delivered via intraperitoneal injection, with the administered volume set at 7.5x the body weight of the mouse, in accordance with the previously mentioned protocol. Blood glucose levels were assessed following a 6-h fasting period and subsequently at 15, 30, 60, 90, and 120 min. Blood glucose measurements were obtained using the Accu-Chek active device.[40] 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.[41] The adipose tissue depots were dissected and weighed individually. The caloric value of weekly intake was analyzed by the amount of food placed in its box and the remaining food.[42] 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. 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

Two centimeters of duodenum was collected for analysis of the depth of the crypts and 2 cm of sigmoid colon for quantification of goblet cells.[43] 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). 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 40× 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).[44] 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′- GTCTCGTGGGC TCGGAGATGTGTATAAGAGACAGGAC - 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. 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).[45] The raw sequence data underwent quality filtering and denoising with DADA2 through the q2-dada2 plugin.[46] To establish a phylogeny, SEPP (Spatially Explicit Phylogenetic Placement) implemented in the q2-fragment-insertion plugin of Qiime2 was employed.[47] All amplicon sequence variants (ASVs) were aligned against the SILVA-138-99 database[48] using the feature-classifier classify-sklearn method, which employed a Naive Bayes classifier[49] 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/.[50] Data were filtered using a minimum count threshold of 5 and a low count filter based on 20% prevalence in the samples. Alpha diversity indices, including observed species, Chao1, Simpson, and Shannon, were assessed. 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.

QUANTIFICATION AND 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 scatterplots, where different groups were represented by distinct colors.[51] 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.[52]