Dietary switch and intermittent fasting ameliorate the disrupted postprandial short-chain fatty acid response in diet-induced obese mice

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Summary

Background Intermittent fasting holds promise as a prevention for obesity, but its effects on established obesity remain uncertain.

Methods We examined two intermittent fasting regimens in diet-induced obese mice, either maintained on a hypercaloric diet or switched to normal chow diet, and compared their effects with those of dietary switch alone (12 mice/group). We assessed metabolic parameters, gut microbiota composition, and fasting and postprandial short-chain fatty acids (SCFAs) levels. Additionally, faecal microbiota was analysed before and after diet-induced weight loss in 18 individuals with obesity.

Findings Intermittent fasting with a hypercaloric diet effectively mitigated weight gain and improved metabolic health–glucose, insulin, total cholesterol, triglycerides, and glucose tolerance ($p < 0.05$) to an extent comparable with dietary switch. Intermittent fasting increased faecal SCFAs levels, especially postprandially (acetate, propionate, and butyrate, $p < 0.05$), mirroring dietary switch. Combining intermittent fasting with dietary switch yielded the greatest improvement in glucose tolerance ($p < 0.01$) and exerted a more pronounced effect on gut microbiota composition (28 significant genera), albeit with reduced SCFA changes. The relative abundance of *Alistipes finegoldii* was associated with postprandial SCFAs levels and/or metabolic parameters in both mice and humans.

Interpretation Intermittent fasting on a hypercaloric diet protects against obesity, while combining intermittent fasting with dietary switch improves glucose metabolism independently of body weight. Both effects are associated with specific microbiota changes and postprandial SCFA dynamics, highlighting the connection between intermittent fasting, microbiota, and metabolic health.

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Keywords: Obesity; Dietary switch; Intermittent fasting; Gut microbiota; SCFAs; Postprandial

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Research in context

Evidence before this study

Caloric restriction, dietary switch and intermittent fasting are widely recognised as promising dietary strategies for managing obesity, though their effectiveness and underlying mechanisms remain unclear. Previous research, primarily based on animal models, has indicated that intermittent fasting may offer metabolic benefits even without weight loss. However, most studies have emphasised intermittent fasting's preventive effects, rather than exploring its potential role in treating established obesity. Furthermore, research on various intermittent fasting regimens has produced inconsistent results, with the outcomes of each intervention varying significantly. As a result, the optimal approach remains unclear. Faecal transplantation and germ-free model studies highlight the gut microbiota's key role in the benefits of dietary interventions, though specific microbes and mechanisms remain unclear. In this context, although short-chain fatty acids (SCFAs) are well-studied microbial metabolites and key candidates mediating these effects, research has primarily focused on SCFA producers or caecal levels, with limited data on circulating SCFAs. Moreover, their postprandial dynamics, which could provide critical insights into microbiota-host metabolic interactions, remain underexplored compared to fasted-state regulation.

Added value of this study

Our research provides valuable insights by emphasising the functionality of the microbiota rather than just its composition, offering a broader understanding of its role in dietary interventions for obesity. We demonstrate that intermittent fasting induces distinct effects on gut microbiota composition, metabolic outcomes, and SCFAs levels, depending on the type of diet. While the impact of intermittent fasting on the intestinal microbial taxonomic composition of a hypercaloric diet was limited, we observed significant effects on microbial functional traits. Notably, although dietary interventions showed a trend toward increased fasting plasma and faecal SCFAs, the most pronounced effects were observed post-feeding. Specifically, we observed that the postprandial SCFA response is disrupted in obesity, and dietary interventions can help restore this metabolic imbalance. Furthermore, postprandial SCFA responses were closely linked to improvements in metabolic parameters. These findings emphasise the critical role of postprandial SCFAs fluctuations in metabolic regulation, highlighting their greater significance compared to fasting levels.

Implications of all the available evidence

Overall, these findings contribute to a better understanding of the mechanisms underlying the therapeutic effects of dietary interventions and reveal opportunities for the development of targeted strategies to combat obesity and associated metabolic conditions. Furthermore, our discoveries shed new light on the potential role of SCFAs in metabolic homoeostasis and underscore the importance of considering the postprandial dynamics of SCFAs in future research.

Introduction

Caloric restriction is considered a promising approach to treating obesity and its complications, but it can be challenging to maintain over the long term. Emerging evidence suggests that diets incorporating intermittent fasting may offer metabolic benefits, even without reduced energy intake and/or weight loss.[1] Dietary switch interventions–where there is a transition from an obesogenic, high-fat diet (HFD) to a healthier, nutritionally balanced diet–have also gained attention as an effective strategy to improve metabolic health and counteract obesity-related disorders.[2–4] However, the molecular mechanisms underlying the metabolic improvements associated with intermittent fasting are not fully understood.

Most animal studies have focused on the potential preventive benefits of intermittent fasting, but its efficacy as a treatment for established obesity remains unclear.[5,6] The most common intermittent fasting regimens in preclinical models of obesity are the intermittent fasting 1:1 regimen and the 2:1 regimen. The intermittent fasting 1:1 regimen, also known as alternate-day fasting, involves one day of feeding followed by one day of fasting. In contrast, the intermittent fasting 2:1 regimen consists of two consecutive days of feeding followed by one day of fasting. However, there is no consensus on the potential beneficial effects of intermittent fasting, and controversial results on energy intake and body weight regulation have been described.[5–9] Indeed, intermittent fasting has been shown to protect against weight gain in most studies,[5,6,8–10] but its effect on weight loss is less clear. It is also uncertain whether these improvements are comparable with those seen with caloric restriction or dietary switch. Therefore, it is of clinical interest to gain a deeper understanding of how food intake, diet type, and fasting time interact to impact metabolic health.

Previous studies have shown that high-fat feeding exerts a dominant impact on both the gut microbiota composition and the host's metabolic phenotype, and that diet composition, rather than obesity itself, primarily drives microbiota structure.[11,12] Moreover, the regulation of the gut microbiota composition has been identified as a key factor in the benefits of intermittent fasting interventions.[8,13–15] However, the specific mechanisms governing the interaction between the gut microbiota and the host remain unclear. The gut microbiota acts as an endocrine organ, converting nutritional cues into hormone-like signals that enter the circulation and travel to distal sites to influence host physiology.[16] The most extensively studied microbial-derived metabolites are the short-chain fatty acids (SCFAs), which are produced by the gut microbiota from the anaerobic fermentation of complex resistant carbohydrates and indigestible polysaccharides. While SCFAs are generally considered beneficial for health,[17,18] some studies have suggested that their overproduction may promote obesity, possibly due to increased energy storage.[19] In the context of intermittent fasting, our understanding of the role of SCFAs remains limited. Preclinical studies have suggested an increase in SCFAs production by intermittent fasting, as demonstrated by the rise in the relative abundance of SCFAs producers[9] or by measuring caecal levels.[8] However, most studies have primarily focused on the metabolic regulation of SCFAs in the fasted state, and there is a gap in our understanding of the postprandial dynamics of these microbial metabolites.[20] After food ingestion, SCFAs secreted into the gut lumen may function as hormone-like metabolites that regulate the enteroendocrine system, including releasing GLP-1.[21] In addition, SCFAs can cross the epithelial barrier and reach peripheral organs via the bloodstream.[22] Indeed, research has shown that some SCFAs can influence adipocyte differentiation and adipokine production,[23,24] regulate hepatic metabolism,[25] and modulate neuronal function.[26] Given that postprandial metabolic dysregulation is recognised as an independent risk factor for non-communicable diseases such as obesity,[27,28] we hypothesised that the postprandial response of SCFAs may be disrupted in obesity. Accordingly, dietary interventions could potentially serve as a means to restore this metabolic imbalance.

Here, we investigated in preclinical models the potential differences between different intermittent fasting regimens (1:1 vs 2:1) once obesity is established. We also questioned whether the potential benefits of intermittent fasting are similar to those of dietary switch and might specifically depend on the diet (intermittent fasting on HFD vs intermittent fasting combined with dietary switch to a normal chow diet [NCD]). To this end, we used a dietary switch as a reference model for weight loss and metabolic recovery in diet-induced obese mice. Furthermore, we aimed to elucidate the underlying mechanisms driving the metabolic benefits of these interventions, focussing in gut microbiota composition and SCFAs metabolism. Our study provides new insights into the role of SCFAs in metabolic regulation.

Methods

Animals

Seventy-two male C57BL/6J mice, aged 7–8 weeks, were purchased from Charles River Laboratories (Barcelona, Spain) and housed at the Faculty of Medicine and Health Science animal facility (Universitat Rovira i Virgili). The mice were maintained under controlled conditions with a 12-h light/dark cycle at 22 °C and were provided *ad libitum* access to food and water. After one week of acclimatisation, all mice were fed a HFD (providing 60% of total calories from fat, 20% from carbohydrates, and 20% from protein; D12492, Research Diets, Brunswick, NJ) *ad libitum* for 8 weeks to promote an obese phenotype. Subsequently, half of the mice were maintained on HFD ($n = 36$), and the other half were switched to a NCD ($n = 36$) (providing 8.4% of total calories from fat, 72.4% from carbohydrate, and 19.3% from protein, SAFE diets A04, Augy, France). Mice from each diet group were then assigned to either *ad libitum* feeding or two intermittent fasting protocols (1:1 and 2:1) for 4 weeks ($n = 12$/group) (scheme in Fig. 1a). Mice were systematically assigned to experimental groups using a cage-based allocation strategy. Each intervention group consisted of two cages (housing six animals per cage), and animals remained in their original cages throughout the study. This cage-based assignment was carefully performed to ensure no significant differences in baseline body weight between groups maintenance of established social hierarchies within cages, and minimisation of stress from cage transfers. The allocation process considered the mean body weight of each cage, distributing cages among treatment groups to achieve balanced baseline characteristics. No animals were moved between cages after the initial obesity induction phase, preserving both the metabolic and social stability of each group. Body weight was monitored weekly during the 4-week dietary intervention, and food intake was recorded daily. At the end of the dietary intervention period, all animals were fasted overnight. To assess both fasting and postprandial metabolic responses, particularly the dynamics of SCFAs, half of the animals in each experimental group ($n = 6$/group) were anesthetised with isoflurane and sacrificed in the fasted state. The remaining animals ($n = 6$/group) were refed with their respective diets for 3 h prior to sacrifice. Blood samples were obtained by cardiac puncture at the time of sacrifice, and faecal samples, adipose tissues, liver, and pancreas were collected.

Humane endpoints were clearly defined and included indicators such as pronounced lethargy, deterioration of coat condition, and reduced responsiveness. Animals were monitored daily, and humane euthanasia was performed when predefined criteria were met, in order to minimise pain and distress. During the study, an unexpected adverse event was observed in one animal from the intermittent fasting 2:1 with dietary switch group. The animal exhibited significant clinical decline, characterised by profound lethargy, an unkempt and coarse coat, and severely reduced responsiveness. In accordance with the established

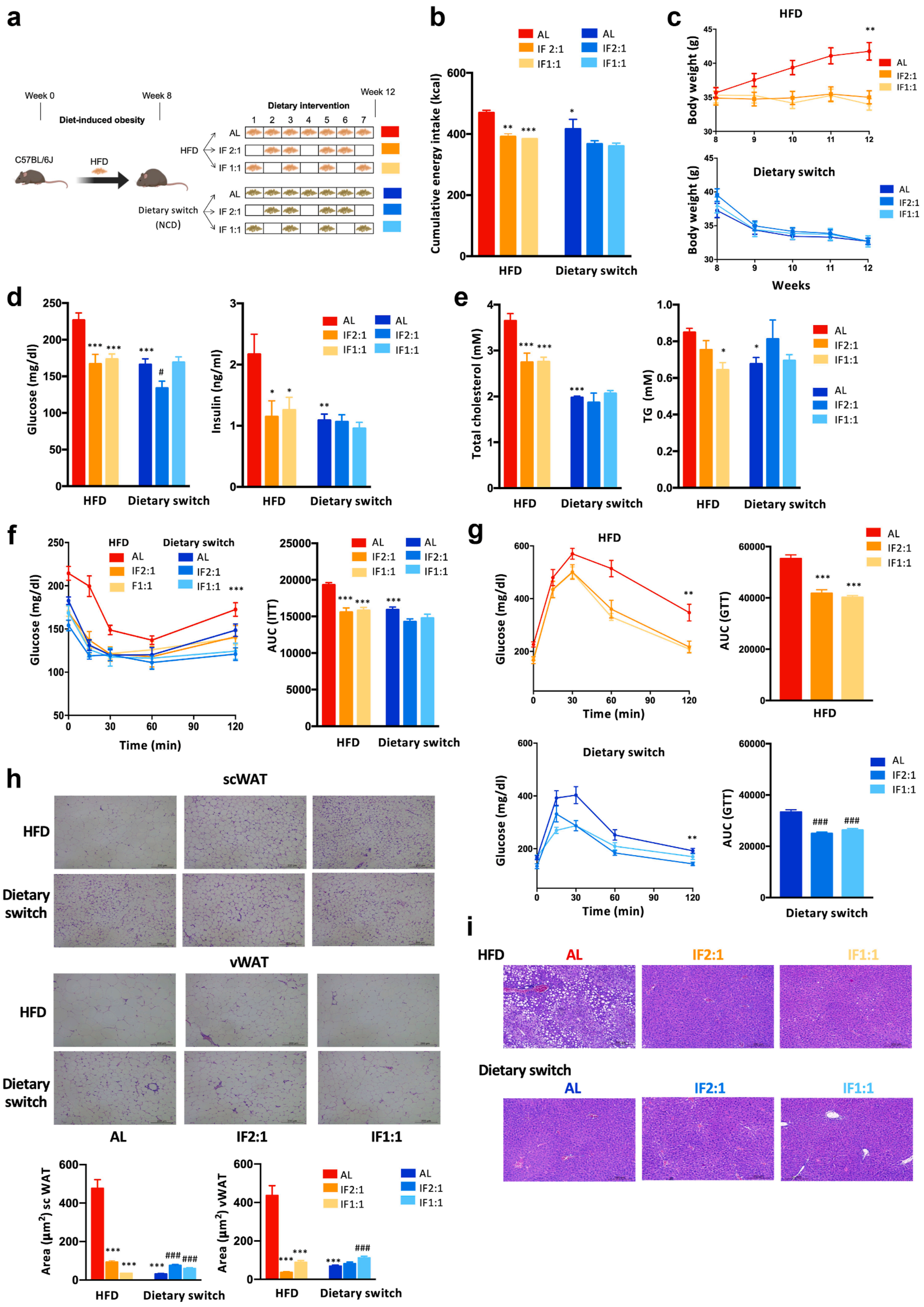

***Fig. 1:* Metabolic effects of different diets in obese mice.** a) Experimental design. Male C57BL/6J mice were fed a high-fat diet (HFD) for 8 weeks. Mice were then maintained on HFD or switched to a normal chow diet (NCD) for 4 weeks. Mice on each diet were randomised to *ad libitum* (AL) feeding or two intermittent fasting (IF) diets (1:1 and 2:1) (n = 11–12/group). b) Cumulative energy intake. c) Body weight

humane endpoint criteria, the animal was promptly and humanely euthanised to prevent further suffering.

Personnel responsible for animal care, follow-up, and feeding were aware of the group allocations. However, all measurements and data analyses were conducted by researchers who remained blinded to the experimental groups throughout the study.

The experimental protocol, including the research question, study design, and analysis plan, was defined within the framework of a project funded by the Instituto de Salud Carlos III (PI20/00095). However, the protocol was not prospectively registered in a public registry.

Biochemical parameters

Plasma insulin levels were measured using a commercial mouse insulin enzyme-linked immunosorbent assay (Mercodia Mouse Insulin ELISA, Uppsala, Sweden, cat# 10-1247-01). Plasma total cholesterol and triglycerides were enzymatically determined using commercial kits on a COBAS 6000 autoanalyzer (Roche Diagnostics, Rotkreuz, Switzerland).

Glucose and insulin tolerance tests

Mice were subjected to a glucose tolerance test (GTT) after a 6-h fast. Glucose was intraperitoneally injected (2 g/kg of body weight), and blood glucose levels were monitored using a glucometer and glucose strips at baseline (time 0) and subsequently at 15, 30, 60, and 120 min post-injection. For the insulin tolerance test (ITT), mice were fasted for 3 h before receiving an intraperitoneal infusion of human insulin (Actrapid, Novo Nordisk, Mainz, Germany, cat# EU/1/02/230/003) at a dose of 0.75 U/kg body weight. Glucose levels were measured before the injection and again at 15, 30, 60, and 120 min afterwards.

Histochemistry

White and brown adipose tissue samples, liver, and pancreas were fixed in 4% formaldehyde, followed by dehydration and paraffin embedding. They were then sectioned, rehydrated, and stained with haematoxylin and eosin (H&E). Following staining, the samples were dehydrated, mounted, and observed under a light microscope.

Faecal microbiome analysis

16S rRNA gene amplicons were obtained following the 16S rRNA gene sequencing Library Nextera XT Illumina protocol (Illumina Inc., San Diego, CA; cat# FC-131-1024). The gene-specific sequences used in this protocol target the 16S rRNA gene V3 and V4 regions. Illumina adaptor overhang nucleotide sequences were added to the gene-specific sequences. The primers were selected as described.[29,30] The full-length primer sequences, using standard IUPAC nucleotide nomenclature, targeting this region were 16S rRNA V3-V4 Forward: TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACGGGNGGC WGC AG and 16S rRNA V3-V4 Reverse: GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GAC TACHVGGG TAT CT AATCC. We used microbial genomic DNA (5 ng/μL in 10 mM Tris, pH 8.5) for the protocol.[30] After 16S rRNA gene amplification, a multiplexing step was performed using the Nextera XT Index Kit (Illumina; cat# FC-131-2001). PCR products (1 μL) were run on a Bioanalyzer DNA 1000 chip to verify the size; the expected size on a Bioanalyzer trace is ~550 bp. After size verification, the libraries were sequenced using a 2 × 300 bp paired-end run (MiSeq Reagent kit v3; cat# MS-102-3003) on a MiSeq Sequencer according to the manufacturer's instructions (Illumina) at the Sequencing and Bioinformatic Service of the FISABIO (Valencia, Spain).

Data were obtained using an *ad hoc* pipeline written in the R Statistics environment version 4.1.0 (R Core Team, 2012), using several open-source libraries, and the sequence data were analysed using the qiime2 pipeline.[31] Meta taxonomy analysis was performed using qiime2 plugins. Denoising, paired-end joining, and chimera depletion were performed starting from paired-end data using the DADA2 pipeline.[32] Taxonomic affiliations were assigned using the Naive Bayesian classifier integrated into the quiime2 plugins. The database used for this taxonomic assignation was the Silva138.[33] Microbial counts were stored through phyloseq (version 1.36.0). The taxa prevalence filter was set at 10%. Alpha diversity was calculated using the Shannon index. Beta diversity at family, genus, and species levels was measured using the Bray–Curtis dissimilarity index and visualised with principal coordinate analysis plots, with ellipses illustrating the 95% confidence regions for the group centroids. The top 20 most abundant species were displayed with relative abundance plots.

Faecal and plasma short-chain fatty acids analysis

Plasma sample preparation: Fifty microlitres of plasma were added to 1.5 mL Eppendorf tubes, followed by the addition of 5 μL of acidified water (15% phosphoric acid

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progression. d) Plasma glucose and insulin levels. e) Total plasma cholesterol and triglyceride (TG) levels (n = 5–6/group). f) Insulin tolerance test (ITT) and area under the curve (AUC) at 12 weeks. g) Glucose tolerance test (GTT) and AUC at 12 weeks. h) Representative images of haematoxylin & eosin staining of subcutaneous and visceral adipose tissue (scWAT and vWAT) sections and quantification of adipocyte area. i) Representative images of haematoxylin & eosin staining of liver sections. All data are presented as mean + SEM. Statistical significance was studied by one- or two-way ANOVA, as appropriate. *p < 0.05, **p < 0.01, ***p < 0.001 vs HFD AL group and #p < 0.05, ###p < 0.001 vs dietary switch AL group.

v/v) and 5 μL of internal standard (300 μM sodium acetate $^{13}C_2$, 60 μM butyric-1,2-$^{13}C_2$ acid, and 30 μM propionic d6 acid), with vigorous mixing. A liquid-liquid extraction step was performed by adding 75 μL of methyl tert-butyl ether (MTBE) and vortexing for 10 min. Tubes were centrifuged at 15,000 rpm for 10 min at 4 °C, and 50 μL samples were transferred into vials with an insert. The vials were centrifuged at 1000 rpm for 30 s at 4 °C, and 1.5 μL was injected into the gas chromatography coupled to triple quadrupole mass spectrometry (GC–MS/MS) instrument.[34]

Faecal sample preparation: Samples (20 mg faecal matter) were placed in a 2 mL Eppendorf tube and mixed with 200 μL of internal standard (6000 μM sodium acetate $^{13}C_2$, 1200 μM butyric-1,2-$^{13}C_2$ acid, and 60 μM propionic d6 acid) and 200 μL of PBS. Samples were vigorously mixed for 10 min and centrifuged at 2500 rpm for 10 min at 4 °C. Supernatants (50 μL) were acidified with 10 μL of 15% phosphoric acid, followed by extracting SCFAs using 1000 μL of MTBE for 10 min. Samples were centrifuged at 1500 rpm for 10 min at 4 °C, and the upper organic layer was transferred to a glass vial for analysis.[34]

GC–MS analysis

SCFAs were separated on a Durabond Free Fatty Acid Phase (DB-FFAP) chromatographic column (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed as follows: (i) initial temperature 40 °C, (ii) linearly raised at 12 °C/min to 130 °C (0 min), (iii) then linearly raised at 30 °C/min to 200 °C (0 min), and (iv) the temperature was ramped at 100 °C/min to 250 °C (4.5 min). The column flow was set at 1.5 mL/min using helium as the carrier gas. The injector was set at 250 °C, and the extracts were injected in splitless mode. Ionisation was carried out by electron impact with an electron energy of 70 eV, and the mass analyser was operated in multi-reaction monitoring (MRM) mode.

Human study

Participants

Twenty-six individuals with preclinical obesity, with a body mass index (BMI) ranging from 30 to 40 kg/m^2, were invited to participate in a prospective, interventional, non-randomised one-arm study (https://doi.org/10.1186/ISRCTN12973246).[35] Inclusion criteria were age >18 and <65 years, absence of acute or chronic systemic disease other than obesity, absence of any pharmacological treatment, and weight stability for at least 3 months before to the study. The study was performed at the University Hospital Joan XXIII from Tarragona (Spain) started in June 2016 and ended in June 2017. All participants received a low-calorie Mediterranean-type diet (20 kcal/kg baseline body weight) comprising 4 daily meals for 6 months (50% carbohydrates, 25–30% lipids, and 20–25% protein) and were encouraged to increase their physical activity. Monthly visits were scheduled with a dietician, including a health status review and diet reminder recommendations. An effective and clinically relevant weight loss was considered when the subjects lost at least 10% of their initial weight at the end of the dietary intervention. Anthropometry, laboratory determinations and metabolic parameters were measured as previously described.[35] Table 1 presents the clinical and laboratory data of a subsample of 18 patients who underwent faecal microbiota analysis.

Stool sample collection, DNA extraction, and metagenomic sequencing

Faecal samples were collected at baseline, and six months after the dietary intervention. We used shotgun sequencing of stool DNA for whole metagenome analysis. Patients collected fresh stool samples at home, which were immediately frozen in their home freezer at −20 °C. Frozen samples were delivered to the hospital within 2 days using insulating polystyrene foam containers and were kept at −80 °C until analysis. DNA extraction and metagenomic sequencing were performed as previously described.[35]

Ethics

All animal studies were supervised and approved by the Universitat Rovira i Virgili Animal Welfare and Governmental Ethics Committee (reference 11425). All experimental procedures involving animals conformed to the European Union Directive 2010/63/EU and the European Commission Recommendation 2007/526/EC on protecting animals used for experimental and other scientific purposes, enacted under the Spanish Royal Decrees 53/2013 and 118/2021. In addition, all animal experiments were conducted in accordance with the ARRIVE guidelines.

The human study was conducted according to the principles of the Declaration of Helsinki and was approved by the ethics committee of University Hospital Joan XXIII (reference C.I. 83/2014). All participants signed a written informed consent before entry into the study. We used the STROBE cohort reporting guidelines.[36]

Statistics

Mouse study

Based on pilot data from the dietary switch group, we anticipated a minimum 5% reduction in body weight. A power analysis using this effect size indicated that 6 mice per group would provide 80% power to detect significant differences ($\alpha = 0.05$). To allow for multiple comparisons and potential animal loss, the sample size was increased to 12 mice per group.

Normality of data distribution was assessed using the Shapiro–Wilk test. For non-normally distributed variables, appropriate transformations were applied to approximate normality prior to statistical analysis. Data for energy intake, body weight, biochemical parameters, adipocyte area, and SCFAs levels were analysed using one-way analysis of variance (ANOVA). Repeated measures Two-way ANOVA was used to study differences in the glucose tolerance test (GTT) and insulin tolerance test (ITT) curves at the intervention endpoint—two-way repeated measures ANOVA was used to compare GTT curves before and after diet intervention within each group. Paired t-tests were conducted to evaluate differences in the weight gain or loss percentage. Spearman correlation analysis was utilised to assess associations between quantitative variables. Regression analyses, employing stepwise forward selection procedures, were carried out to identify the predictive value of postprandial response of SCFAs on clinical parameters. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS (version 28.0, SPSS Inc., Chicago, IL) or GraphPad Prism software (version 8.0, GraphPad Inc., La Jolla, CA). Graphical representations were generated with GraphPad Prism 8 Software.

Regarding faecal microbiota analyses, alpha diversity was measured with the Shannon diversity index and compared between groups by the non-parametric Mann–Whitney test. Beta diversity was quantified with the Bray–Curtis dissimilarity metric, represented with a principal coordinate analysis (PCoA) and the first two dimensions were compared between groups with a PERMANOVA. Differential abundance analysis between groups was conducted at both genus and species level with the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) method, adjusting for weight. p-values were adjusted with the Holm method and considered statistically significant at $q < 0.05$. Statistical analyses and graphical representations were generated with R software (version 4.3.2) and the libraries vegan (v2.6-4), microbiome (v1.24.0), MicrobiomeStat (v1.1.3), ANCOMBC (v2.4.0), mia (v1.10.0), phyloseq (v1.46.0), BiodiversityR (v2.15-4), ComplexHeatmap (v2.18.0), and ggpubr (v0.6.0).

Variables	Pre	Post	p-value
n	18	18	–
Sex (n, %)	Men 6 (33.3) Women 12 (66.6)	Men 6 (33.3) Women 12 (66.6)	–
Age (years)	48.2 ± 11.6	48.7 ± 11.5	ns
BMI (kg/m^2)	35.4 ± 3.9	31.4 ± 3.6	**<0.0001**
Waist (cm)	108.8 ± 8.9	100.3 ± 9.3	**<0.0001**
Fasting glucose (mmol/L)	5.2 ± 0.6	4.5 ± 0.4	**0.002**
Fasting insulin (pmol/L)	104.6 ± 34.3	42.8 ± 16.9	**0.009**
Homa-IR	4.0 ± 1.4	2.0 ± 1.2	**0.006**
AUC glucose	781.6 ± 163.7	737.8 ± 121.3	ns
HbA1c (%)	5.5 ± 0.4	5.4 ± 0.4	**0.027**
HbA1c (mmol/mol)	36 ± 4	32 ± 4	**0.027**
Total cholesterol (mmol/L)	4.96 ± 0.63	4.47 ± 0.66	**0.019**
HDL-cholesterol (mmol/L)	1.23 (1.12–1.57)	1.26 (1.03–1.38)	ns
LDL-cholesterol (mmol/L)	3.06 ± 0.46	2.71 ± 0.47	**0.013**
Triglycerides (mmol/L)	1.08 (0.9–1.34)	1.02 (0.7–1.16)	ns

Data are presented as mean ± SD or median (25th–75th percentiles), based on Shapiro normality-test evaluation. Glucose response to a meal tolerance test is expressed as Area-under the Curve (AUC). p values for the normal distributed variables were calculated using paired t-test; for the non-normal distributed variables, Wilcoxon signed rank test was used. Statistically significant clinical variables were identified with a p-value lower than predefined threshold of 0.05. Abbreviations: AUC, area under the curve; HDL, high density lipoprotein; LDL, low density lipoprotein; ns, non-significant.

Table 1: **Main anthropometric and metabolic variables of human study.**

Human study

A formal sample size calculation was not performed as this study utilised pre-existing biological samples from our previously published cohort.[35] The original study was powered to detect clinically relevant differences in primary outcome measure, with $\alpha = 0.05$ and 80% power, and the available sample size ($n = 18$) was deemed adequate for the current secondary analyses of comparable metabolic parameters.

The Shapiro–Wilk test was used to assess the normality of data distribution. For normally distributed variables, paired t-tests were applied, while non-normally distributed variables were analysed using the Wilcoxon signed-rank test. Clinical variables were considered statistically significant at a predefined threshold of $p < 0.05$. Spearman correlation analysis was performed to assess associations between variables. Statistical analyses were conducted using SPSS (version 28.0, SPSS Inc., Chicago, IL) or GraphPad Prism (version 8.0, GraphPad Inc., La Jolla, CA), and graphical representations were generated with R software (version 4.3.2).

Role of funders

The funders had no role in the design of the study, data collection, data analysis, or manuscript writing.

Results

Intermittent fasting on a hypercaloric diet attenuates diet-induced obesity and improves metabolic health, similar to dietary switch

The investigation of different dietary approaches in murine models of obesity, including intermittent fasting and dietary switch, is essential to gain a full understanding of their potential therapeutic effects and to optimise their application in the treatment of obesity-related disorders.

We investigated the interplay between food intake, diet type, and intermittent fasting regimen in diet-induced obese mice exposed to different dietary interventions. Mice were subjected to different intermittent fasting protocols (2:1 and 1:1) and diet compositions (HFD and NCD) over a 4-week period (Fig. 1a). As expected, obese mice switched from HFD to NCD, dietary switch, exhibited a 13% decrease in cumulative energy consumption over the 4-week period compared with the group freely fed a HFD (Fig. 1b). A comparable decrease in cumulative energy consumption was observed in obese mice on an HFD subjected to intermittent fasting (Fig. 1b). By contrast, combining intermittent fasting with dietary switch had no significant effect on energy intake compared to obese mice subjected to dietary switch alone (Fig. 1b). A significant influence of intermittent fasting on weight regulation was observed in mice on the HFD, as they did not show the expected weight gain (Fig. 1c, upper panel).

Conversely, obese mice subjected to dietary switch showed an immediate and sustained weight loss not further affected by the inclusion of intermittent fasting (Fig. 1c, lower panel). Furthermore, while dietary switch improved fasting plasma glucose and insulin levels, intermittent fasting regimens also had a beneficial effect, particularly in the context of the HFD (Fig. 1d). A similar trend was observed for cholesterol and triglyceride levels (Fig. 1e) and insulin sensitivity (Fig. 1f). Analysis of glucose homoeostasis showed that intermittent fasting improved glucose tolerance in mice on both diets (HFD and NCD), as assessed by a GTT (Fig. 1g). The beneficial effects of intermittent fasting in the context of a hypercaloric diet, HFD, may have been influenced by the fact that the analyses were performed at the end of the intervention, when the body weights were clearly different, and comparisons were made between different groups. Therefore, we assessed the effects of intermittent fasting before the intervention (week 8) and during the follow-up period (1 month after the intervention) within each respective group. Analysis of glucose tolerance revealed the beneficial effects of intermittent fasting on both diets (HFD and NCD) (Supplementary Figure S1a), indicating that intermittent fasting on a hypercaloric diet exerts metabolic improvements even in the absence of significant weight loss (Supplementary Figure S1b). At the tissue level, the positive effects of intermittent fasting regimens were also evident in the context of an HFD. Both 2:1 and 1:1 regimens significantly reduced the size of white adipocytes in both subcutaneous and visceral depots. However, they did not heighten the effect of dietary switch on this tissue (Fig. 1h).

Similarly, examination of liver tissue showed that dietary switch almost wholly reversed the hepatic steatosis induced by HFD, which was also observed with intermittent fasting in the presence of HFD (Fig. 1i). No significant differences were found in the pancreas or brown adipose tissue (Supplementary Figure S2). Overall, the results indicate that dietary switch and intermittent fasting on a HFD have comparable efficacy in protecting obese mice from further deterioration. In this respect, dietary switch might be the most effective therapy for weight loss, and the combination of intermittent fasting with dietary switch to treat glucose intolerance.

The effect of intermittent fasting on the gut microbiota depends on the type of diet and protocol used and is related to glucose homoeostasis

Given the central role of the gut microbiota in host metabolism, we compared the effects of different intervention strategies on the gut microbiome. The analysis revealed no significant differences in the richness of the gut microbiome (Shannon alpha diversity) (Fig. 2a). Contrastingly, beta diversity (a composition parameter) showed changes at multiple taxonomic levels, including family, genus, and species (Fig. 2b). Notably, the groups of mice on the HFD showed greater proximity to each other (Fig. 2b) when compared with mice subjected to dietary switch. This suggests that intermittent fasting induced a distinct restructuring of the microbiome depending on diet composition, with 1:1 as the most divergent group. As expected, many distinct genera were noted when comparing the HFD *ad libitum* and dietary switch groups (Fig. 2c, first column). Analysis of the impact of intermittent fasting on the gut microbiome revealed a greater number of distinct genera showing changes in relative abundance in mice fed a NCD, particularly under the IF1:1 regimen (Fig. 2c, second and third columns). This trend was consistent with the beta diversity analysis (Fig. 2b). However, when applied to a HFD, intermittent fasting changed a limited number of genera (Fig. 2c, fourth and fifth columns).

At the species level, the analysis identified seven species whose abundance was changed by the type of dietary intervention (Fig. 2d). Notably, four of these species (*Parabacteroides goldsteinii*, *Bacteroides dorei*, *Alistipes finegoldii*, and *Dubosiella newyorkensis*) were among the top 20 taxa (Supplementary Figure S3). Obese mice subjected to dietary switch showed a significant increase exclusively in the relative abundance of *B. dorei*. Both intermittent fasting protocols implemented on a HFD increased the relative abundance of *P. goldsteinii* and *B. dorei*, accompanied by a decrease in the relative abundance of *Clostridium leptum* and *D. newyorkensis* (Fig. 2d). Intermittent fasting induced different changes depending on the protocol when applied to a NCD. In the case of 2:1 protocol, *B. dorei* decreased while *Alistipes indistinctus* and *C. leptum* increased. Conversely, 1:1 protocol increased the relative abundance of *B. dorei*, *A. finegoldii*, and *Ileibacterium valens* (Fig. 2d). These results again support the notion that the effects of intermittent fasting on the gut microbiota differ depending on the diet composition and the intermittent fasting protocol used.

In light of these findings, we investigated whether changes in these species might be associated with some of the observed metabolic changes. *A. finegoldii* and *I. valens* showed inverse correlations with body weight, glucose, and area under the curve (AUC) of GTT and ITT, whereas *D. newyorkensis* and *P. goldsteinii* showed

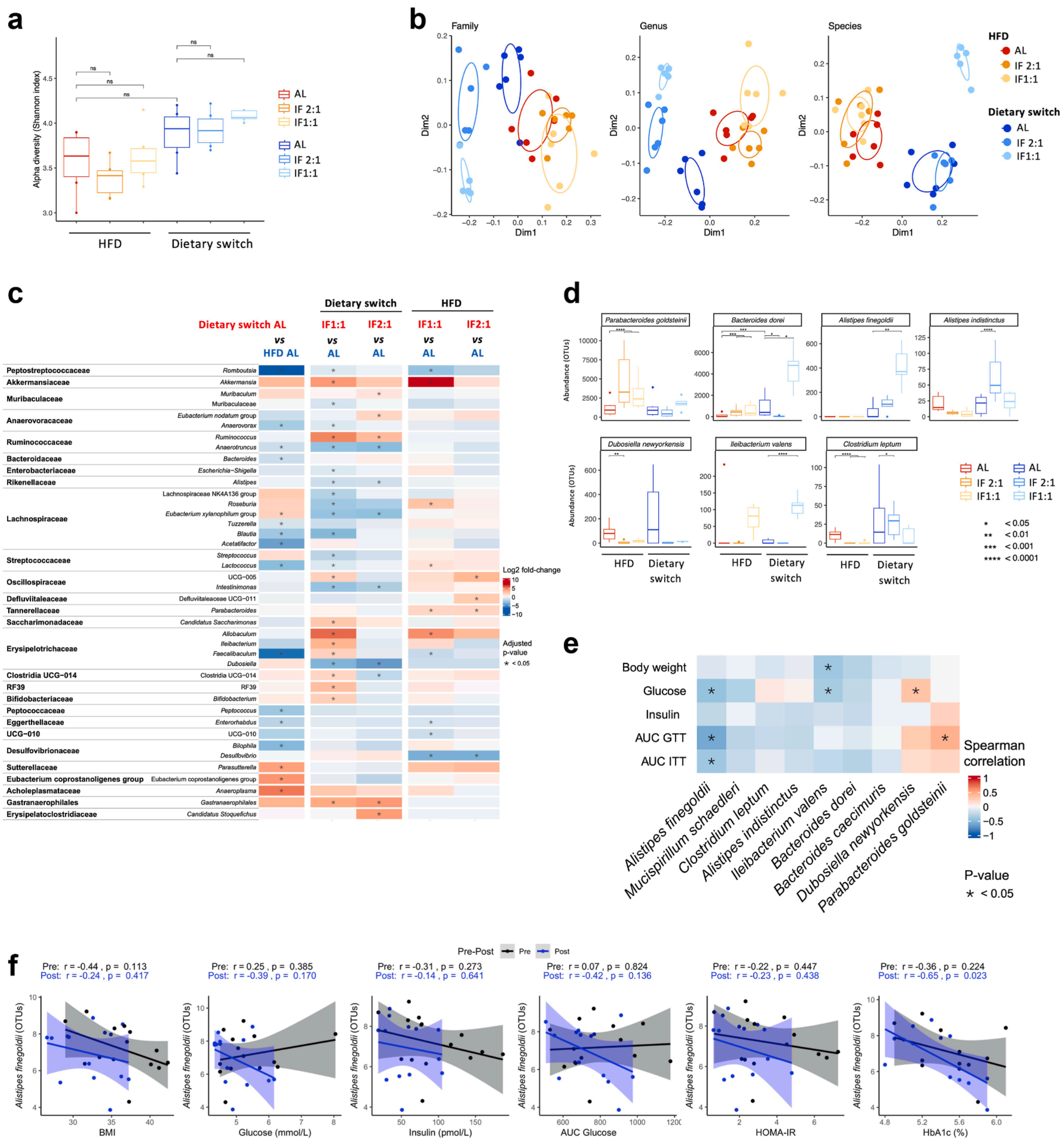

***Fig. 2:* Intermittent fasting restructures the gut microbiome depending on diet and fasting protocol.** a) Alpha diversity. b) Beta diversity visualised with principal coordinate analysis (PCoA) plots. c) Heatmap of genera. d) Differential abundance for species. e) Spearman correlations with clinical parameters. (n = 35, 5–6/group). f) Spearman correlations of *Alistipes finegoldii* with clinical parameters in individuals with preclinical obesity (n = 14). *p < 0.05, **p < 0.01, ***p < 0.001 (two-tailed unpaired t-test, one- or two-way ANOVA or PERMANOVA test, as appropriate). Abbreviations: HFD, high-fat diet; AL, *ad libitum*; IF, intermittent fasting.

positive associations with glucose and AUC of GTT (Fig. 2e). Taken together, these results suggest that the 1:1 protocol, especially when combined with dietary switch, induces the most significant changes in the gut microbiota. Of note, the increased relative abundance of *A. finegoldii* and *I. valens* by this dietary intervention might be partly responsible for the improved metabolic outcomes, particularly in glucose homoeostasis.

To evaluate the potential influence of *A. finegoldii* on glucose metabolism in humans, we analysed the faecal gut microbiota of 18 participants with preclinical obesity before and after an effective weight loss dietary intervention. Notably, a significant inverse correlation between the relative abundance of *A. finegoldii* and HbA1c values, particularly after weight loss (Fig. 2f). These results, consistent with our preclinical observations, underscore a potentially significant role of *A. finegoldii* in regulating glucose homoeostasis.

Fasting plasma SCFAs levels show stronger associations with metabolic parameters than do faecal SCFAs levels

To understand how dietary interventions affect gut microbiota and glucose homoeostasis, we focused on SCFAs. Increasing SCFAs levels through diet or supplementation has been proposed to counteract the metabolic dysregulation in obesity.[22] However, the effect of metabolic disorders on circulating and faecal SCFAs levels is still controversial,[37] and studies on dietary interventions are limited.[38] We therefore investigated the influence of diets, particularly those strongly influencing the gut microbiome (IF1:1 under both dietary switch and HFD), on the main SCFAs (acetate, propionate, and butyrate). We first assessed faecal levels in fasted mice, which revealed a significant increase in faecal acetate only in obese mice undergoing intermittent fasting on a HFD (Fig. 3a). Also, significantly increased levels of faecal butyrate were observed following dietary switch (Fig. 3a). By contrast, no effects of intermittent fasting on faecal SCFAs levels were observed in mice on the NCD (Fig. 3a). We then examined correlations between faecal SCFAs levels, and the positive outcomes observed with the diets. We found negative correlations between faecal propionate and body weight, as well as between faecal butyrate and both total cholesterol and the AUC of the GTT (Fig. 3b).

Given the hypothesis that systemic SCFAs levels are better indicators of metabolic health than faecal SCFAs,[21] we repeated this analysis using plasma SCFAs. The results showed a significant increase in plasma acetate levels only in obese mice subjected to dietary switch (Fig. 3c). Notably, higher levels of plasma SCFAs were associated with less body weight (for plasma acetate) and better glucose tolerance and lipid profile (for plasma propionate and butyrate) (Fig. 3d).

Thus, despite the lack of a consistent effect of diet interventions on fasting faecal and systemic SCFAs levels, the results suggest an association between higher plasma SCFAs levels and improved metabolic health.

Improvement in postprandial responses for SCFAs is associated with metabolic health and increased relative abundance of *A. finegoldii*

Given that SCFAs may primarily act in response to dietary intake, an often overlooked aspect, we shifted our focus to investigating the postprandial response of SCFAs.

We first examined the postprandial faecal response in lean mice, observing a significant increase in the

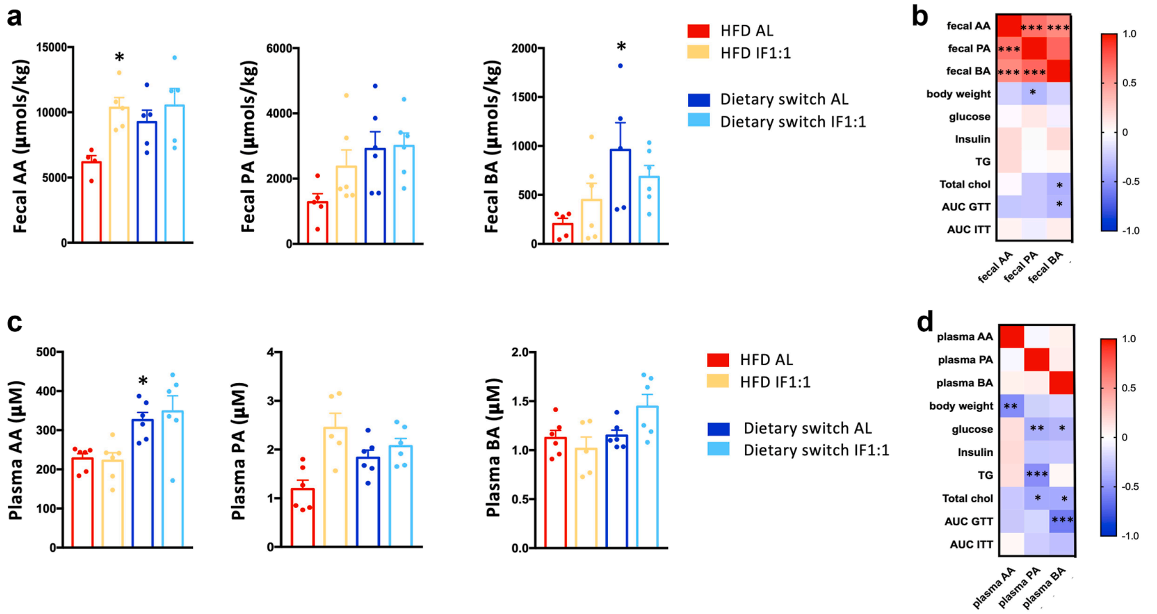

***Fig. 3*: Fasting levels of short-chain fatty acids in obese mice after dietary interventions.** Analysis of short-chain fatty acids (SCFAs) in plasma and stool samples of mice subjected to a 16-h fast. a) Faecal levels of acetate (AA), propionate (PA), and butyrate (BA). b) Correlation heatmap of body weight, metabolic parameters, and faecal AA, PA, and BA. c) Plasma levels of AA, PA, and BA. d) Correlation heatmap of body weight, metabolic parameters, and plasma AA, PA, and BA. Statistical significance was studied by one-way ANOVA. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs HFD AL group. Data are presented as mean + SEM (a and c) and Spearman correlation coefficients (b and d) (n = 5–6/group). Abbreviations: HFD, high-fat diet; AL, *ad libitum*; IF, intermittent fasting.

levels of all tested SCFAs following food intake (Fig. 4a, indicated by the green bar). This effect was suppressed entirely in diet-induced obese mice (Fig. 4a, indicated by the red bar). Of note, all diet interventions restored the postprandial SCFA response at the faecal level, albeit with some variability. Specifically, intermittent fasting resulted in a heightened postprandial faecal butyrate response in both diets, whereas increased responses for acetate and propionate were observed with dietary switch and intermittent fasting only under HFD conditions (Fig. 4a).

A more complex pattern was observed at the circulating SCFAs level. Specifically, acetate and butyrate levels decreased following food consumption in lean mice (Fig. 4b), contrasting with the elevated levels observed in faecal samples. Conversely, and consistent with the faecal response, propionate levels increased after food intake in lean mice, but this meal-related response was disrupted in obese mice (Fig. 4b, middle panel). Notably, only obese mice undergoing dietary switch showed restoration of the postprandial plasma propionate response (Fig. 4b).

Next, we investigated the relationships between postprandial responses of SCFAs and metabolic parameters. Faecal postprandial responses for all SCFAs showed negative correlations with body weight and parameters related to glucose homoeostasis, including glucose, insulin, AUC of GTT, and AUC of ITT (Fig. 4c). Regarding postprandial plasma responses, meal-induced plasma propionate levels were also associated with better metabolic health (Fig. 4d). We performed linear regression analyses to investigate the potential impact of postprandial SCFAs dynamics on metabolic regulation. The results indicated that the postprandial responses of faecal acetate influenced several metabolic parameters, including final body weight, fasting glucose, fasting insulin, glucose tolerance, and insulin sensitivity. Additionally, fasting glucose levels depended on feeding-induced plasma propionate (Table 2). These findings suggest that the postprandial changes in SCFAs may provide valuable insights into the metabolic responses to dietary interventions beyond the significance of fasting SCFAs levels alone. Of note, the metabolic adaptability to SCFAs appeared to be compromised in the context of obesity, but dietary interventions hold promise to restore this.

Finally, we performed correlation analyses to question whether postprandial SCFAs levels were associated with the species modulated by diet interventions. The relative abundance of *A. finegoldii*, which was highly increased under the 1:1 intermittent fasting protocol combined with a dietary switch (Fig. 2d), showed significant positive correlations with both faecal and plasma levels of the tested SCFAs (Fig. 4e). By contrast, *D. newyorkensis* and *P. goldsteinii* species were negatively associated with some plasma and faecal SCFAs (Fig. 4e).

Discussion

By studying and comparing different dietary approaches in a murine model of diet-induced obesity, our study provides valuable insights into the complex interactions between food intake, diet type, and intermittent fasting regimen. Specifically, our findings highlight the benefits of intermittent fasting, in the presence or absence of a hypercaloric diet, in mitigating diet-induced obesity and improving metabolic health, comparable to a dietary switch. Moreover, intermittent fasting combined with a dietary switch provided better outcomes regarding glucose homoeostasis and had greater effects on the gut microbiota. These findings contribute to a better understanding of the mechanisms underlying dietary interventions' therapeutic effects and may help manage obesity and related metabolic diseases more effectively.

To date, only one preclinical study has examined the effects of intermittent fasting (protocol 1:1) with either HFD or a low-fat diet in diet-induced obese mice,[5] and no study has compared the effects of different diets and intermittent fasting regimens. In line with to the results of Gotthardt and colleagues,[5] we found that dietary switch and intermittent fasting helped prevent weight gain on a high-calorie diet. However, compared with the pre-diet intervention in the same mice, dietary switch induced greater weight loss than intermittent fasting on a hypercaloric diet despite similar cumulative energy intake. Interestingly, intermittent fasting on an HFD showed improved glucose tolerance even without weight loss.

To assess the efficacy of different dietary interventions in established obesity, we examined the metabolic health of obese mice subjected to different dietary regimens over a 4-week period. Fasting glucose, lipid profile, glucose tolerance, insulin sensitivity, and liver and adipose tissue showed similar improvements in obese mice after intermittent fasting on a hypercaloric diet compared to a dietary switch. These findings align with Herderson et al.'s research, which revealed similar outcomes in body weight and glucose homoeostasis between intermittent fasting and caloric restriction interventions.[6] Conversely, intermittent fasting combined with dietary switch resulted in improvements primarily in glucose tolerance compared with dietary switch alone, as observed by Gotthardt et al. in obese mice subjected to intermittent fasting on a low-fat diet.[5] Interestingly, our results indicate that both intermittent fasting protocols produced comparable effects on metabolic health. This suggests that the frequency of fasting periods may not be the primary determinant of metabolic outcomes, at least in our experimental setting. Our study underscores the potential of intermittent fasting on a hypercaloric diet, dietary switch, or intermittent fasting combined with dietary switch as therapeutic strategies for managing established obesity and emphasises the importance of

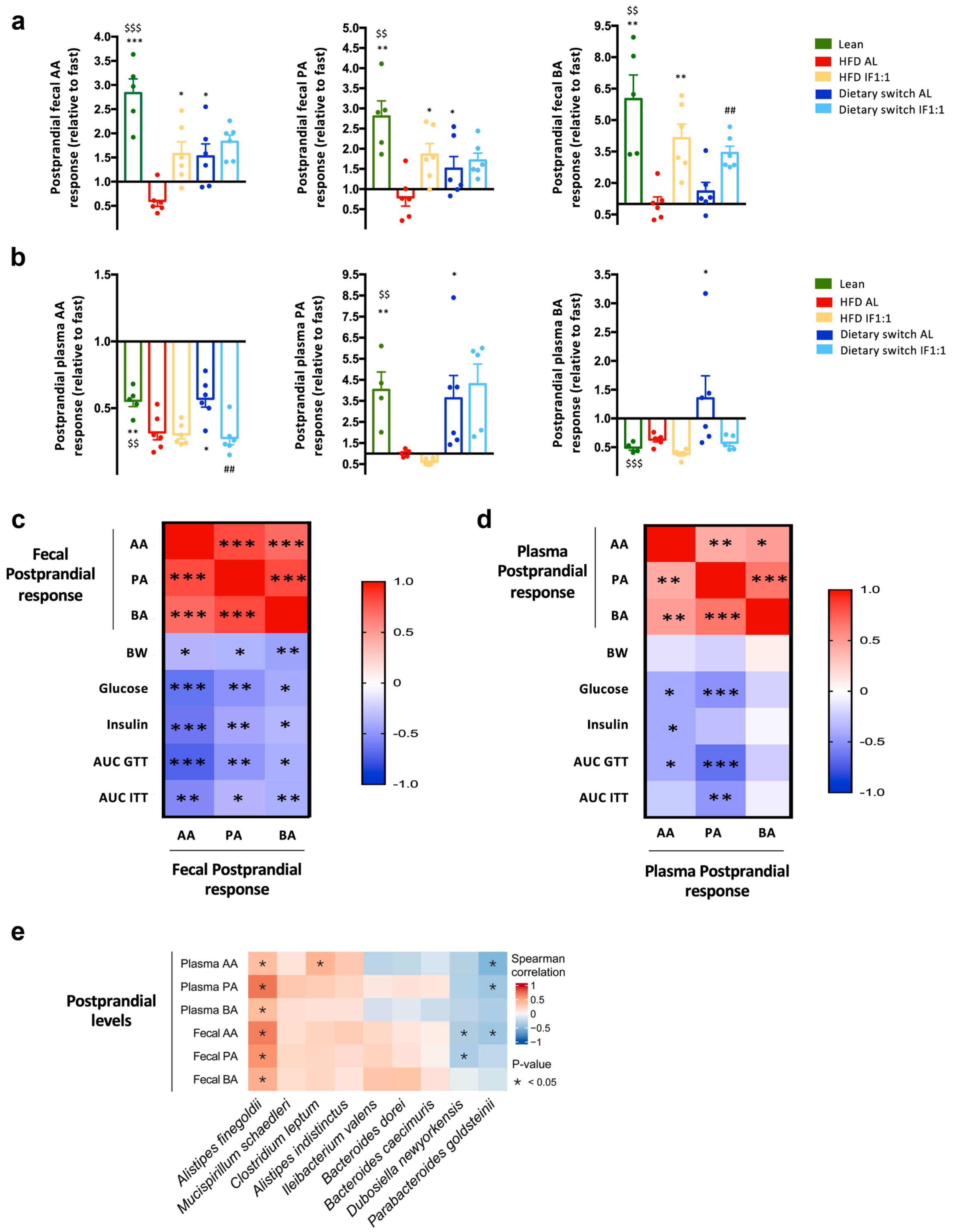

Fig. 4: **Postprandial response of short-chain fatty acids in lean and obese mice and after dietary interventions.** Analysis of short-chain fatty acids (SCFAs) in plasma and stool samples of mice subjected to a 16-h fast and subsequently fed for 3 h. a) Faecal levels of acetate (AA), propionate (PA), and butyrate (BA). b) Plasma levels of AA, PA, and BA. c) Correlation heatmap of body weight, metabolic parameters, and faecal postprandial response. d) Correlation heatmap of body weight, metabolic parameters, and plasma postprandial response. e) Correlation heatmap of postprandial levels of plasma and faecal and species. Statistical significance was studied by one-way ANOVA. $$p < 0.01, $$$p < 0.001 vs fast lean group. *p < 0.05, **p < 0.01, ***p < 0.001 vs HFD AL group and ##p < 0.01 vs dietary switch AL group. Data are presented as mean + SEM (a, b) and Spearman correlation coefficients (c–e) (n = 4–5 for lean mice, n = 35 for obese mice, 5–6/group). Abbreviations: HFD, high-fat diet; AL, *ad libitum*; IF, intermittent fasting.

Body weight	**R = 0.456; R^2 = 0.208**				
variable	β (non-standardised)	SE	95% CI	β (standardised)	p-value
Constant	36.698	1.091	34.484–38.915	–	<0.001
Faecal postprandial response of AA	−2.056	0.688	−3.455 to 0.657	−0.456	0.005
Glucose	**R = 0.626; R^2 = 0.391**				
variable	β (non-standardised)	SE	95% CI	β (standardised)	p-value
Constant	229.637	14.092	200.967–258.308	–	<0.001
Faecal postprandial response of AA	−30.361	9.227	−49.133 to 11.590	−0.464	0.002
Plasma postprandial response of PA	−2.916	1.321	−5.603 to 0.229	−0.312	0.034
Insulin	**R = 0.531; R^2 = 0.282**				
variable	β (non-standardised)	SE	95% CI	β (standardised)	p-value
Constant	1.661	0.157	1.341–1.982	–	<0.001
Faecal postprandial response of AA	−0.353	0.098	−0.552 to 0.153	−0.531	0.001
AUC of GTT	**R = 0.697; R^2 = 0.486**				
variable	β (non-standardised)	SE	95% CI	β (standardised)	p-value
Constant	56008.933	3585.519	48722.281–63295.584	–	<0.001
Faecal postprandial response of AA	−12830.692	2262.627	−17428.905 to 8232.480	−0.697	<0.001
AUC of ITT	**R = 0.545; R^2 = 0.297**				
variable	β (non-standardised)	SE	95% CI	β (standardised)	p-value
Constant	20209.511	1214.418	17732.690–22686.332	–	<0.001
Faecal postprandial response of AA	−2859.129	789.670	−4469.671 to 1248.587	−0.545	0.001

Variables included in the regression models based on significant correlations from the correlation analysis. For body weight: Faecal postprandial responses of AA, PA, and BA. For glucose: Faecal postprandial responses of AA, PA, and BA, and plasma postprandial responses of AA and PA. For insulin: Faecal postprandial responses of AA, PA, and BA, and plasma postprandial response of AA. For AUC of GTT: Faecal postprandial responses of AA, PA, and BA, and plasma postprandial responses of AA and PA. For AUC of ITT: Faecal postprandial responses of AA, PA, and BA, and plasma postprandial response of PA. Abbreviations: AA, acetate; PA, propionate; BA, butyrate; AUC, area under the curve; GTT, glucose tolerance test; ITT, Insulin tolerance test; SE, standard error; CI, confidence interval.

***Table 2:* Linear regression models showing associations between body weight and glucose metabolism parameters with the postprandial response of faecal and plasma levels of SCFAs in mice.**

individualised approaches tailored to specific metabolic goals.

The mechanisms underlying the metabolic improvements associated with dietary interventions are complex. The gut microbiota is considered an important mediator of the interactions between dietary regimens and host metabolism.[8,9,39–42] Although many studies have been conducted, the effects of dietary interventions on animal gut microbiota remain incompletely understood, with inconsistent and inconclusive findings regarding the regulation of individual bacterial strains or groups (reviewed by Purdel, 2023[43]). Furthermore, evidence from studies using different animal models and biospecimens suggests that distinct fasting regimens may affect the microbiota differently.[44] However, caution must be exercised in interpreting these results, and, to our knowledge, no studies have compared intermittent fasting across different regimens and dietary compositions in established obesity. To shed light on the complex interplay between dietary interventions, gut microbiota composition, and metabolic outcomes, we examined the gut microbiota of obese mice under defined conditions. It is widely suggested that the gut microbiome undergoes dynamic changes in response to various dietary interventions.[45] However, our findings indicate that intermittent fasting on a hypercaloric diet, while significantly affecting metabolic health, failed to induce substantial changes in the faecal microbiota. At the genus taxa level, intermittent fasting on an HFD resulted in a lower number of significantly different genera when compared with dietary switch. Only three genera, *Romboutsia*, *Faecalibaculum*, and *Enterorhabdus*, were consistently reduced in relative abundance under intermittent fasting 1:1 condition, both on a HFD and NCD. Interestingly, all 3 have been reported as obesity-related genera,[46–48] and *Romboutsia* has been proposed as a potential predictive biomarker of human obesity-related metabolic abnormalities.[49]

Surprisingly, intermittent fasting combined with dietary switch had the greatest effect on gut microbiota composition despite not showing a more substantial effect on metabolic outcomes. Furthermore, the specific intermittent fasting protocol used had different effects on faecal microbiota, with IF1:1 having greater effects on NCD and HFD. These findings, together with those of a previous study in lean mice,[45] highlight the importance of investigating the interplay between diet composition, food intake, and intermittent fasting protocol. This is essential to better understand the therapeutic and mechanistic potential of dietary interventions in treating established obesity.

Focussing on the genera consistently enriched across all interventions and reported as beneficial bacteria,[50] the genus *Akkermansia* was significantly increased in relative abundance in IF1:1 under both hypercaloric and NCD conditions. Regarding those genera whose relative abundance were consistently suppressed across all interventions and associated with obesity-related phylotypes,[46] *Romboutsia* significantly decreased in relative abundance following IF1:1 on HFD, IF1:1 combined with dietary switch and dietary switch alone. Additionally, *Anaerotruncus*, *Bacteroides*, and *Peptococcus* were decreased after dietary switch alone or combined with intermittent fasting but not in response to other interventions. The genus *Alistipes* was particularly interesting, which was significantly suppressed only under the combined intermittent fasting and dietary switch intervention. Recent evidence has highlighted *Alistipes* dysbiosis and its association with various non-communicable diseases.[51] This might suggest that a shift in *Alistipes* partially contributes to the improved glucose tolerance observed specifically with these dietary interventions. Indeed, we found that the relative abundance of *A. finegoldii* was significantly increased in obese mice following the combination of intermittent fasting and dietary switch, particularly under 1:1 protocol. This increase was associated with improvements in fasting glucose levels, glucose tolerance, and insulin sensitivity. Similarly, in our study involving participants with obesity, we observed a significant negative correlation between *A. finegoldii* and Hb1ac values following a diet-induced weight-loss intervention. These results align with previous findings demonstrating that dietary changes can rapidly and consistently alter the composition and function of the human gut microbiota, highlighting its responsiveness to nutritional interventions.[52] Therefore, our findings are in line with the potential therapeutic effects of *A. finegoldii*, as it has been previously demonstrated by its ability to ameliorate insulin resistance in obese mice.[53]

Intermittent fasting-induced microbial shifts have been reported to directly contribute to white adipose tissue beiging and mitigate obesity-related disturbances.[8] Along these lines, we observed increased *Ucp1* mRNA levels with intermittent fasting, albeit only in combination with dietary switch and not on a hypercaloric diet (Supplementary Figure S4). Thus, given the metabolic improvements observed with intermittent fasting on a hypercaloric diet, it is plausible that mechanisms beyond gut microbiota composition may underlie the observed metabolic outcomes. Several studies have suggested that intermittent fasting and caloric restriction exert beneficial effects on health through changes in microbiota composition, including the enrichment of anti-inflammatory-related microorganisms, and changes in the metabolic function of the gut microbiota, particularly pathways related to carbohydrate, lipid, and amino acid metabolism.[8,41,54–58]

Microbial metabolites, including SCFAs, play a crucial role in metabolic homoeostasis by regulating various physiological processes, including the intestinal barrier, immune function, and feeding behaviour.[37,59] While there is debate regarding the impact of metabolic diseases on circulating and faecal levels of microbial metabolites, some studies suggest that intermittent fasting may increase their production.[8,43,60,61] In the present study, we observed significant increases in fasting plasma and faecal levels of some SCFAs, specifically acetate and butyrate, though these changes were limited to specific interventions and were not observed across all experimental groups. Notably, systemic SCFAs levels, particularly propionate and butyrate, appeared to be more closely associated with metabolic health than faecal levels, as previously proposed.[62] We examined their postprandial dynamics to explore the impact of SCFAs on metabolic balance. We observed that faecal SCFAs levels increased following food consumption in lean mice, a pattern disrupted in obese mice. This is in accordance with studies in which postprandial caecal and portal vein SCFAs were increased after a meal in healthy chow-fed rats but not in HFD-fed obese rats.[20] Intriguingly, we found that intermittent fasting on a hypercaloric diet restored this regulation similar to dietary switch, although it did not significantly impact gut microbiota composition. Furthermore, the increased faecal postprandial response correlated with improved metabolic health, with the postprandial response of faecal acetate identified as the primary determinant of glucose homoeostasis. Regarding plasma SCFAs levels, the postprandial levels of acetate and butyrate showed a similar reduction, as observed in humans.[63] However, we noted a postprandial increase in propionate plasma levels in lean mice, a response disrupted in obese counterparts, and only the dietary switch restored this regulation. Additionally, associations with metabolic health were predominantly observed with the plasma postprandial response of propionate. Increased propionate levels after feeding were specifically linked to improved glucose metabolism, consistent with previous research in rodents that highlights the beneficial effects of propionate supplementation on glucose homoeostasis.[64–66] *Alistipes* has emerged as a potential SCFAs producer,[51] and we found a significant positive correlation between *A. finegoldii* and postprandial levels of all plasma and faecal SCFAs. This finding is consistent with previous literature indicating that *A. finegoldii* can produce acetate and propionate *in vitro*[51] and possesses genomic features compatible with SCFA biosynthesis pathways. Overall, these results shed new light on the role of SCFAs in metabolic homoeostasis and emphasise the importance of considering their postprandial behaviour.

Nevertheless, there are several limitations in our study that should be acknowledged. First, we used a NCD instead of a purified control diet for comparison with the HFD, which may introduce confounding nutritional differences beyond fat content. This limitation should be considered when interpreting the metabolic and microbiota outcomes observed in the dietary switch model. Second, while our results suggest a potential role for *A. finegoldii* as an SCFA-producing bacterium, further mechanistic studies are needed to establish whether it causally contributes to the observed increases in *SCFAs* and the associated metabolic improvements. Experiments such as fecal slurry assays, *in vivo* supplementation, or fecal microbiota transplantation could help evaluate these causal effects. Third, our microbiota data are based on 16S rRNA gene sequencing, which provides relative abundances.

In conclusion, our findings highlight the potential benefits of intermittent fasting on a hypercaloric diet. While HFD is typically associated with adverse effects, our study demonstrates that integrating intermittent fasting into such diets can prevent weight gain and promote metabolic improvements comparable to a dietary switch. These benefits are likely mediated through the regulation of postprandial microbial metabolites, particularly SCFAs. However, the most optimal outcomes in weight management and glucose homoeostasis are observed with combined intermittent fasting and a dietary switch, which also induces the most significant changes at the microbiota level.

Given the different mechanisms underlying the therapeutic effects of intermittent fasting and dietary switch, personalised interventions must be designed for specific metabolic targets. Our present findings provide a roadmap for developing targeted methodologies for obesity and associated metabolic disorders. Furthermore, our findings highlight the need to consider the postprandial dynamics of SCFAs when studying the functionality of the microbiota as producers of metabolites crucial for energy homoeostasis.

Contributors

V.C.-M.: conception and design of the study, sample collection, experimental planning and conduction, manuscript writing, and financial support. M-M.R.-P., T.V.-C., L.C., A.M.-B., E.B., C.N.-R., and L.S.-P.: technical and animal procedure assistance. J.B.: faecal microbiome data analysis. F.J.O.-P.: patient data analysis. R.B.: histological examination. S.P.: clinical study execution. S.F.-V. and J.V.: conception and design, financial support, administrative support, data analysis and interpretation, manuscript writing, and final approval of the manuscript. The authors read and approved the final manuscript.

Data sharing statement

All relevant data is included in the article, further enquiries can be directed to the corresponding authors. The metagenome datasets from this study are available in the EBI European Nucleotide Archive (ENA) under accession studies PRJEB43147 and PRJEB76433, with accession numbers ERX5107074–ERX5107109 and ERS20224254–ERS20224289.

Declaration of interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105827.

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