The benefits of switching to a healthy diet on metabolic, cognitive and gut microbiome parameters are preserved in adult rat offspring of mothers fed a high-fat, high-sugar diet

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Key words

cafeteria diet, cognition, diet switch, gut microbiota, high fat diet, maternal obesity

Abstract

Scope: Maternal obesity increases the risk of health complications in children, highlighting the need for effective interventions. We used a rat model of maternal obesity to examine whether a diet switch intervention could reverse the adverse effects of an unhealthy postweaning diet.

Methods and results: Male and female offspring born to dams fed standard chow or a high-fat, high-sugar ‘cafeteria’ (Caf) diet were weaned onto chow or Caf diets until 22 weeks of age, when Caf-fed groups were switched to chow for 5 weeks. Adiposity, gut microbiota composition and place recognition memory were assessed before and after the switch. Body weight and adiposity fell in switched groups but remained significantly higher than chow-fed controls. Nonetheless, the diet switch improved a deficit in place recognition memory observed in Caf-fed groups, increased gut microbiota species richness and altered β diversity. Modelling indicated that adiposity most strongly predicted gut microbiota composition before and after the switch.

Conclusion: Maternal obesity did not alter the effects of switching diet on metabolic, microbial or cognitive measures. Thus, a healthy diet intervention led to major shifts in body weight, adiposity, place recognition memory and gut microbiota composition, with beneficial effects preserved in offspring born to obese dams.

1. Introduction

Maternal obesity is associated with a range of pregnancy and birth complications, and with an increased risk of metabolic diseases and behavioural changes in children [1-4]. These effects have been linked to inflammatory, epigenetic and structural brain changes [5] and altered gut microbiome composition [6-10]. There is, therefore, a need to identify effective interventions in mothers and children.

A recent clinical trial found that a dietary education intervention moderately reduced weight gain in pregnant women with obesity [11]. Work in animal models has shown that switching mothers to a healthy diet prior to gestation [12-16] or during gestation and/or lactation can reduce the adverse metabolic and behavioural effects of maternal obesity on offspring [14, 17-19], though extended diet exposure prior to intervention may prevent beneficial effects [20]. Moreover, switching adult rodents from a diet high in fat and/or sugar to regular chow can improve diet-induced metabolic [21-23] and cognitive [24-26] impairments, with some exceptions [27]. However, it is not known whether a healthy diet in offspring can reduce the effects of an unhealthy diet consumed earlier in life, or the diet consumed by their mothers.

The present study examined this question using a rat model in which offspring of mothers fed a healthy chow diet (Chow) or an unhealthy, cafeteria-style, high-fat/high-sugar diet (Caf) were weaned onto the same diet (groups CC and CafCaf) or switched to the other diet (groups CCaf and CafC). At 22 weeks of age, rats in the ChowCaf and CafCaf groups were switched to the chow diet for a further 5 weeks. Whole-body adiposity, faecal microbiota composition and short-term recognition memory were assessed pre- and post-switch.

2. Experimental Section

Animals and diets

This study conformed to the Australian code for the care and use of animals for scientific purposes 8th edition (2013) and was approved by the Animal Care and Ethics Committee of UNSW Sydney (approval #19/74A). Adult female (*N*=36) and male (*N*=16) Sprague Dawley rats, obtained from Animal Resources Centre (Perth, Australia) were group-housed in a colony room maintained at 18-22°C on a 12-hour light/dark cycle (lights on 3:00 am–3:00 pm). Standard chow (Specialty Feeds®, 14 kJ/g; 59% carbohydrate, 26% protein, 15% fat) and water were continuously available. The Caf diet consisted of a range of high-fat, high-sugar commercially available foods eaten by people. Foods were varied daily, with chow, water and 10% sucrose solution always available, as described previously [28]. Food intake over 24-h was measured weekly on a per-cage basis.

Maternal obesity cohort

Full details of this cohort are reported in our previous study [29]. Briefly, females were fed Chow (n=15, mean weight = 213 ± 2 g [SEM]) or Caf (n=21, 217 ± 1 g) diets for 6 weeks, then mated with chow-fed males. Litters were standardised to 6 male and 6 female pups, where possible. As reported previously, there were no differences in litter size between chow (mean [SEM] = 14.7 [1.6] pups) and Caf (14.8 [2.9]) diet groups [29]. Maternal diets were continued throughout gestation and lactation. At P20, male and female siblings (2-3/sex/litter) were weaned onto chow or Caf diets, forming four offspring diet groups, that were housed with same-sex littermates. After studying earlier timepoints [29], the present study reports data from 51 male and 42 female offspring that were retained for further study and not reported on elsewhere. Rats were re-housed into group-matched cages at 14 weeks of age (2-4/cage) and then continued on their postweaning diets until 22 weeks of age.

Diet switch intervention

Figure 1 shows the 4 groups in this study: offspring from chow mothers fed chow (group CC; 17 male, 14 female); offspring from chow mothers fed Caf (group CCaf; 16 male, 13 females); offspring from Caf mothers fed chow (group CafC; 9 male, 8 female); and offspring from Caf mothers fed Caf (group CafCaf; 9 male, 7 female). At 21 weeks of age, faeces were collected and whole-body adiposity (EchoMRI-900, BRIL, UNSW) and place recognition memory were assessed. At 22 weeks of age, groups CCaf and CafCaf were switched to chow diet, while groups CC and CafC remained on chow. Body weight and 24-h energy intake were assessed twice per week after the switch. Faecal collection, adiposity and place recognition measures were repeated in the week prior to endpoint in week 27.

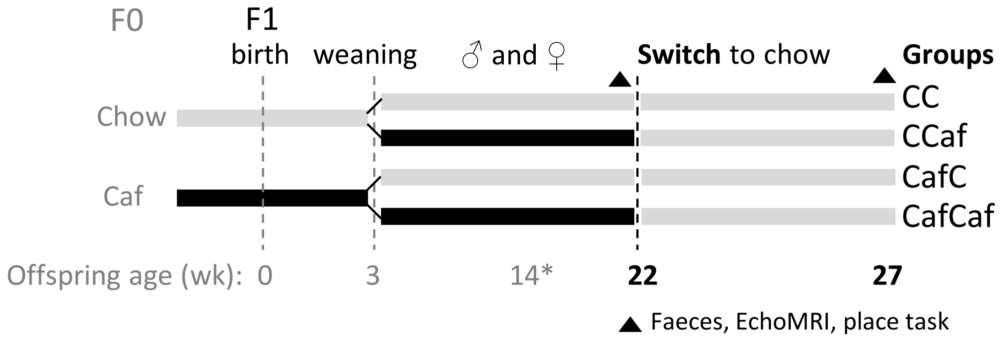


Figure 1. Experimental timeline. Male and female rat siblings born to chow- or Caf-fed mothers were weaned onto chow or Caf diets, forming four groups. At 22 weeks of age, groups fed Caf diet postweaning were switched to chow for 5 weeks, with group differences in gut microbiome composition, adiposity and place recognition memory assessed pre- and post-switch. *Data from siblings reported previously [29].

Place recognition memory test

Place recognition memory was assessed in a black acrylic square arena (60×60×60cm), using objects that varied in material and shape (bottles, mugs and cans), with object positioning within the maze counterbalanced within groups. Rats were habituated to the empty arena prior to the first test. Rats were placed in the arena with two identical objects (5-min; familiarization phase), then returned to the home-cage while arena and objects to be cleaned with 50% ethanol (5-min; retention phase), followed by a 3-min test where one object was moved to a novel location while the other remained in its original location. The proportion of total exploration time spent exploring the object moved to a novel location was used as a measure of recognition: if rats remember the original locations, they preferentially explore the object in the new location. Data were scored using Macropod ODlog software by an experimenter ‘blind’ to group allocation.

Endpoint measures

At week 27 (5-weeks post-switch), non-fasted rats were deeply anaesthetized (ketamine/xylazine, i.p.) and body weight, girth, and nasoanal length were measured. Blood was collected by cardiac puncture and rats were immediately decapitated. Liver and retroperitoneal fat pads were weighed and snap-frozen. Plasma was stored at -30^{o}C for analysis of leptin and insulin (CrystalChem Inc., Chicago, IL, USA) and triglyceride content (Roche triglyceride reagent, Sigma glycerol standard).

Faecal DNA extraction and 16S rRNA amplicon sequencing

After EchoMRI measures, faeces were gently extracted into a sterile tube and immediately frozen on dry ice. Faecal DNA was extracted (PowerFecal DNA Isolation Kit, Qiagen) according to the manufacturer’s instructions. After measuring DNA concentration and quality (DeNovix DS-11 Spectrophotometer, DeNovis, Inc., Delaware, USA), samples were stored at -80^{o}C. Composition of the microbial communities was assessed by Illumina amplicon sequencing (2 x 250 bp MiSeq chemistry, V4 region, 515F-806R primer pair; Ramaciotti Centre for Genomics, UNSW Sydney) using a standard protocol. Sequence data were analyzed using MOTHUR [30], which included removal of ambiguous bases and homopolymers longer than 15 base pairs, alignment with SILVA database, chimera checking with UCHIME, classification against the RDP Ribosomal Database training set (version16_022016), and removal of singletons. Sequences were clustered into operational taxonomic units (OTU) at 97% nucleotide identity to generate an OTU table with the taxonomy and number of sequences per OTU in each sample. Commands were derived from MiSeq SOP [31] and modified as required. Sequence data were subsampled to n = 18,968 total clean reads/sample.

Data analysis

Effects of the diet switch on body weight, body composition and place recognition were assessed in mixed-model ANOVAs with maternal diet (chow or Caf), postweaning diet (chow or Caf) and sex (male or female) as between-subjects factors and switch (pre- and post-switch) as the within-subject factor. Gut microbiota α diversity parameters (Margalef's species richness, Pielou's species evenness and Shannon index) were assessed by Permutational Multivariate of Analysis of Variance (PERMANOVA) using a Euclidean distance resemblance matrix on subsampled count data, with sex included as a covariate. OTU tables were then standardised by dividing feature read counts by total number of reads in each sample to calculate relative abundances. Standardised data were then square root transformed and inter-sample resemblances calculated using Bray-Curtis similarities. All statistical analyses examined sex-specific differences in the offspring. Non-metric Multi-dimensional Scaling (NMDS) plots, Permutational Multivariate Analysis of Variance (PERMANOVA) and Permutational Analysis of Multivariate Dispersions (PERMDISP) were applied on Bray-Curtis resemblance matrices. Analyses were conducted using PRIMER v7 (Primer-e Ltd., Plymouth, United Kingdom) [32]. Constrained ordination using distance-based redundancy analysis (dbRDA) was performed for identification of individual OTUs associated with host variables. This enabled the quantification of the contribution of host variables to each dbRDA axis. The relationships of the top 100 OTUs to a dbRDA axis of interest was assessed using Spearman correlations, with the Benjamini-Hochberg procedure used to correct for False Discovery Rate. Figures were generated in GraphPad Prism and PRIMER. Results are expressed as mean ± SEM and were considered significant at $p<0.05$.

3. Results

Energy intake

As shown in Figure 2, the day before the switch, energy intake was significantly higher in Caf-fed offspring (*F*=68.09, *p*<.01), and was approximately 1.8 times and 2.3 times higher in males and females, respectively. Energy intake was significantly higher in male than in female cages (*F*=40.68, *p*<.01), with no effect of maternal obesity and no interaction (largest *F*=1.18, *p*=.29). Energy intake fell markedly in rats switched from Caf to chow and remained lower than rats maintained on chow, but steadily increased over time. Analyses confirmed a significant time x postweaning diet interaction (*F*=49.26, *p*<.01), and significant main effects of postweaning diet (*F*=132.29, *p*<.001) and sex (*F*=215.61, *p*<.001). Despite increasing over time, chow intake remained significantly lower in switched groups relative to the non-switched chow groups each week after the switch (all *F*>16.57, *p*<.01, Bonferroni correction).

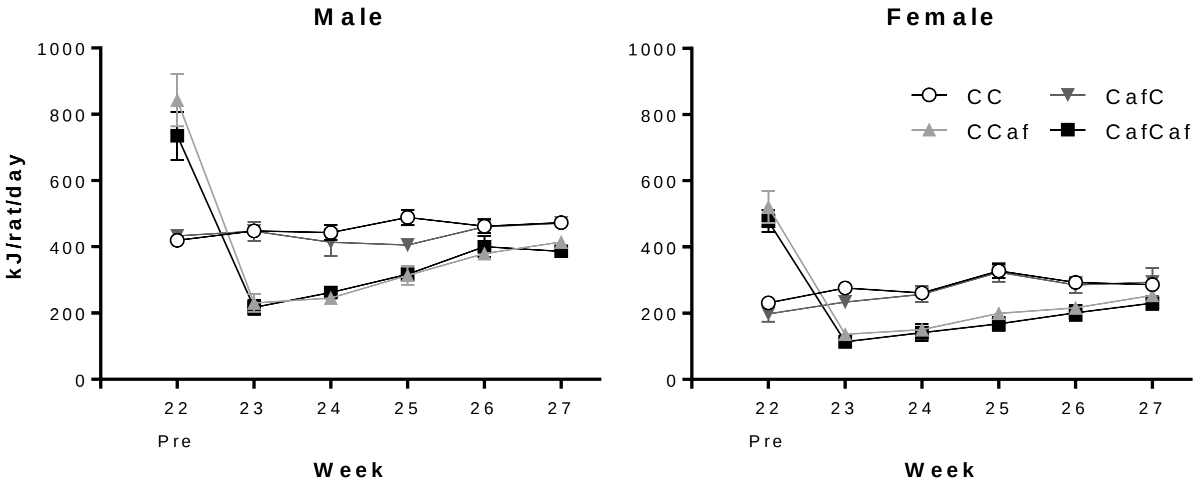


Figure 2. Energy intake (kJ/rat/day) in cages of male and female offspring switched from Caf to chow diet at 22 weeks of age (CCaf and CafCaf groups) or continued on chow (CC and CafC groups) until 27 weeks of age. Offspring were born to mothers fed chow or Caf diets. Data were analysed by mixed ANOVA with factors of maternal diet (chow or Caf), postweaning diet (chow or Caf), sex and time (6 measures). Group

CC: offspring from chow dams weaned onto chow (no switch, open circle); CCaf: offspring from chow dams weaned onto Caf (switch, grey triangle); CafC: offspring of Caf dams weaned onto chow (no switch, inverted triangle); CafCaf: offspring from Caf dams weaned onto Caf (switch, solid square).

Body weight

As shown in Figure 3, body weight before the switch was significantly higher in groups fed Caf diet (*F*=151.43, *p*<.001) and in male rats (sex main effect: *F*(1, 85) = 474.08, *p*<.001) with no significant maternal diet effects (all *F*<1). Groups switched from Caf to chow diet lost weight steadily after the switch, while those maintained on chow continued to gain weight. This trend was confirmed by a significant postweaning diet x switch interaction (*F*=519.19, *p*<.001), with no other significant main or interaction effects, apart from a main effect of sex (*F*=543.29, *p*<.001). Male and female rats switched from Caf to chow remained ~ 20% heavier than chow-fed counterparts in week 27 (males: *F*= 28.83, *p*<.01; females: *F*=31.94, *p*<.01).

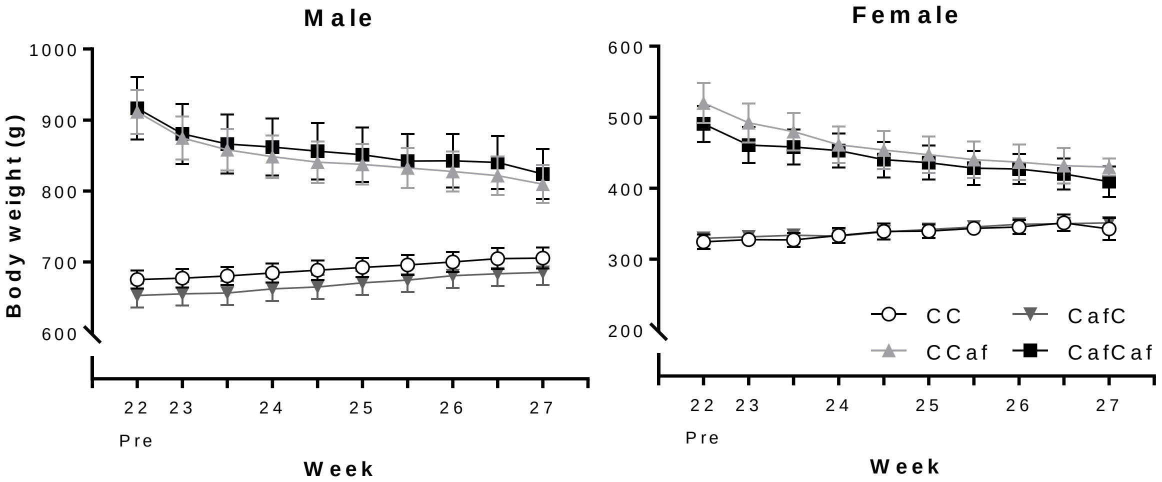


Figure 3. Body weight of male (n=51) and female (n=42) offspring switched from Caf to chow diet at 22 weeks of age (CCaf and CafCaf groups) or continued on chow (CC and CafC groups) until 27 weeks of age. Offspring were born to mothers fed chow or Caf diets. Data were analysed by mixed ANOVA with factors of maternal diet (chow or Caf), postweaning diet (chow or Caf), sex, and time (10 measures). Group CC:

offspring from chow dams weaned onto chow (17 male, 14 female, no switch, open circle); CCaf: offspring from chow dams weaned onto Caf (16 male, 13 female, switch, grey triangle); CafC: offspring of Caf dams weaned onto chow (9 male, 8 female, no switch, inverted triangle); CafCaf: offspring from Caf dams weaned onto Caf (9 male, 7 female, switch, solid square).

Body composition

Figure 4 shows fat mass, as a percentage of body weight, before and after the diet switch. Pre-switch fat mass was significantly higher in offspring fed Caf diet (*F*=342.58, *p*<.001) and in females than in males (*F*=22.86, *p*<.001), with a significant postweaning diet x sex interaction (*F*=13.72, *p*<.001) indicating a greater effect of postweaning Caf diet in females than males. Post-switch, fat mass significantly decreased in Caf-fed offspring switched to chow, with a greater reduction in females, while remaining stable in groups continued on chow, supported by a 3-way interaction between switch, postweaning diet and sex (*F*=38.27, *p*<.01) and 2-way interactions between switch and postweaning diet (*F*=826.88, *p*<.001) and switch and sex (*F*=25.24, *p*<.01). There were no significant maternal diet effects (largest *F*=1.92, *p*=.17). Despite these changes, post-switch fat mass remained significantly higher in offspring fed Caf diet postweaning (*F*=62.88, *p*<.001) and in females than in males (*F*=7.74, *p*=.007), with no significant effects of maternal diet (all *F*<2.34, *p*=.13).

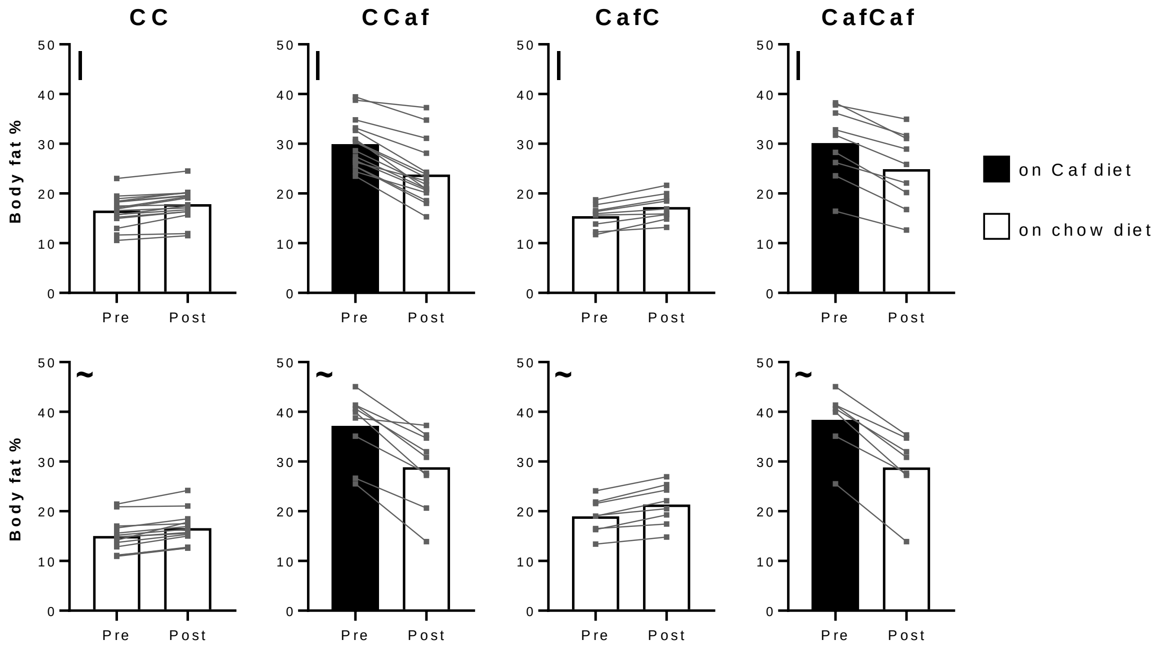


Figure 4. Percent body fat of male (n=51) and female (n=42) offspring switched from Caf to chow diet at 22 weeks of age (CCaf and CafCaf groups) or continued on chow (CC and CafC groups) until 27 weeks of age. Offspring were born to mothers fed chow or Caf diets. Data were analysed by mixed-ANOVA with factors of maternal diet, offspring diet (Chow or Caf) and time (2 measures). Group CC: offspring from chow dams weaned onto chow (17 male, 14 female, no switch); CCaf: offspring from chow dams weaned onto Caf (16 male, 13 female, switch); CafC: offspring of Caf dams weaned onto chow (9 male, 8 female, no switch); CafCaf: offspring from Caf dams weaned onto Caf (9 male, 7 female, switch).

Place recognition memory

Figure 5 shows place recognition data in each group pre- and post-switch (left) alongside the overall effect of the diet switch (right). Pre-switch, place recognition was significantly lower in groups fed Caf than chow (*F*=5.72, *p*=.019), with no other significant main or interaction effects (largest *F*(=1.54, *p*=.22). Analysing the change in place recognition from pre- to post-switch revealed a significant switch x postweaning diet interaction (*F*=4.32, *p*=.041), with no other main or interaction effects (largest *F*=1.61, *p*=.21). To clarify the interaction, subsequent analyses found that place recognition significantly improved in offspring switched from Caf to chow (*F*=4.77, *p*=.035) with no change in rats maintained on chow (*F*<1) (Fig. 5, right). At post-switch testing, one male CafCaf rat failed to explore objects and was excluded. There were no significant main or interaction effects of maternal diet, offspring diet or sex at the post-switch test (largest *F*=2.41, *p*=.124). Total exploration time was significantly higher in females (24.9 +/- 1.2s [SEM]) than males (mean: 19.1 +/- 1.1s) (*F*=12.71, *p*=.001), but there were no other significant main or interaction effects (largest *F*=2.08, *p*=.153).

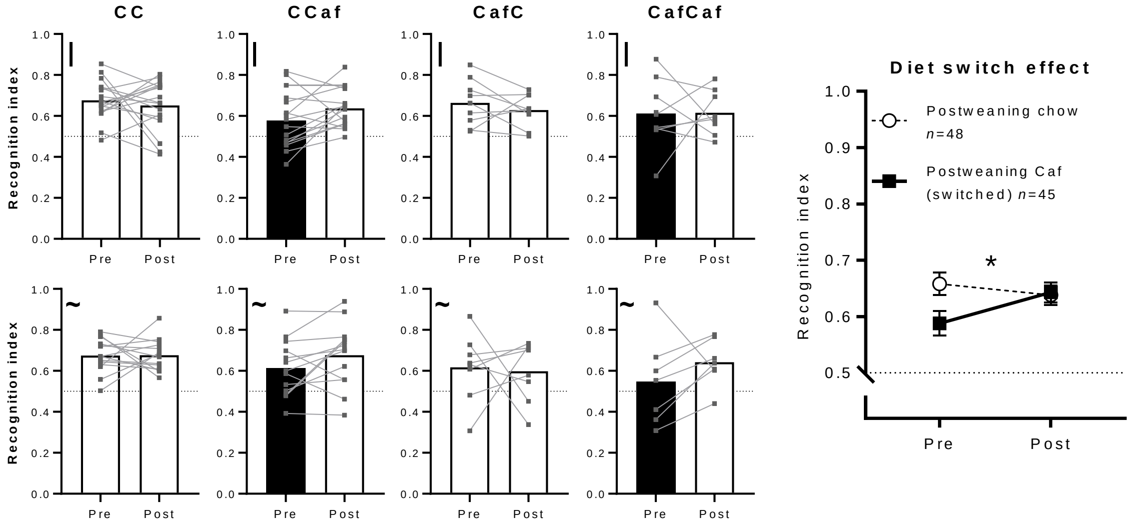


Figure 5. Place recognition memory in male (n=51) and female (n=42) offspring before (Pre) and after (Post) a switch from Caf to chow diet at 22 weeks of age (CCaf and CafCaf groups) or continued access to chow (CC and CafC groups). Offspring were born to mothers fed chow or Caf diets. Left: plots of individual group data. Right: plot showing significant postweaning diet x time interaction, collapsed across maternal diet and sex. Data analysed by mixed ANOVA with factors of maternal diet, offspring diet and time. Group CC: offspring from chow dams weaned onto chow (17 male, 14 female, no switch); CCaf: offspring from chow dams weaned onto Caf (16 male 13 female, switch); CafC: offspring of Caf dams weaned onto chow (9 male, 8 female, no switch); CafCaf: offspring from Caf dams weaned onto Caf (9 male, 7 female, switch). **p*<.05, diet x time interaction. Dotted line at y= 0.5 marks equal exploration of both objects; values > 0.5 indicate greater exploration of the object moved to a novel location.

Endpoint measures

Supplementary Table 1 shows endpoint measures, which were analysed in 2x2x2 ANOVAs (maternal diet x offspring diet x sex). Relative to chow-fed offspring, groups weaned onto Caf diet and switched to chow in week 22 remained significantly heavier, and exhibited greater girth, retroperitoneal fat and liver mass, and nasoanal length. Plasma leptin was significantly higher but blood glucose was significantly lower in offspring fed Caf diet postweaning, with no differences in plasma insulin or triglycerides. Significant sex main effects (usually, males > females) were found for all measures except plasma triglycerides. No maternal diet main effects were significant (all *p* > .10).

Gut microbiota α diversity

Supplementary Figure S1 shows the relative abundance of the main phyla before and after the diet switch intervention. As shown in Table 1 and Figure 6, postweaning Caf diet exposure significantly reduced species richness and Shannon diversity, but not evenness, while the diet switch significantly increased species richness. There were no main effects or interactions involving maternal diet. In offspring born to chow-fed mothers, postweaning Caf diet reduced richness and Shannon diversity, prior to the switch (i.e., group CCaf<CC, *p*<.05 and *p*<.001, respectively). Analysis of within-group changes found that the diet switch significantly increased species richness (*p*<.01) and Shannon diversity (*p*<.05) within group CCaf, and species richness in group CC (*p*<.05). Post-switch, there were no differences between these groups (both *p* > .05; see Fig. 6). In offspring from Caf-fed mothers (groups CafC and CafCaf), there no significant differences in species richness prior to the switch, no significant within-group changes in alpha diversity parameters, and no significant differences post-switch (all $p > 0.05$).

[Table 1 here]

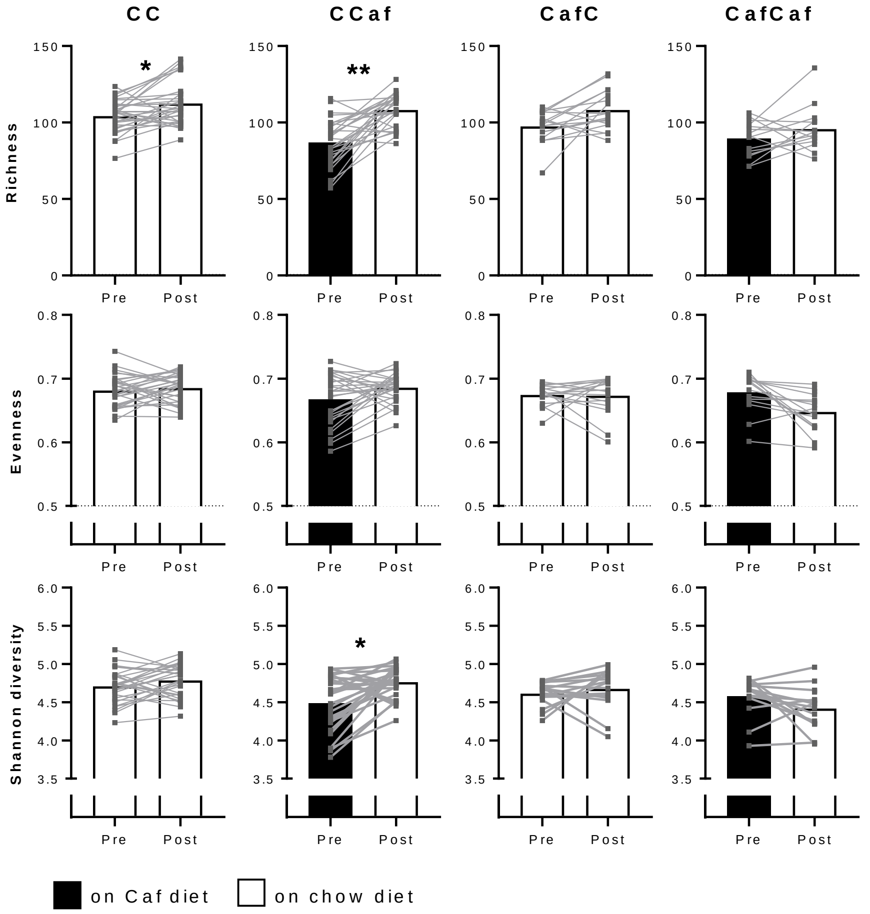


Figure 6. Gut microbiota alpha diversity parameters in offspring before (Pre) and after (Post) a switch from Caf to chow diet at 22 weeks of age (CCaf and CafCaf groups) or continued access to chow (CC and CafC groups). Offspring were born to mothers fed chow or Caf diets. PERMANOVA was used to assess effects of maternal diet and Postweaning diet using Euclidian resemblance matrix. Data are displayed as mean ± SEM. Group CC: offspring from chow dams weaned onto chow (17 male, 14 female, no switch); CCaf: offspring from chow dams weaned onto Caf (16 male, 13 female, switch); CafC: offspring of Caf dams weaned onto chow (9 male, 8 female, no switch); CafCaf: offspring from Caf dams weaned onto Caf (9 male, 7 female, switch). *p<0.05, **p<.01 for within-group change from pre- to post-switch.

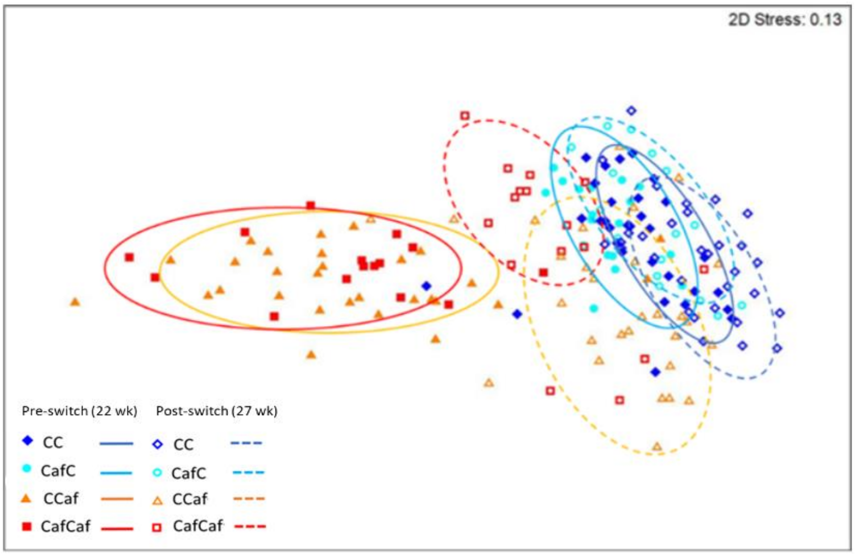
Gut microbiota β diversity

PERMANOVA analyses of gut microbiota composition revealed significant effects of the diet switch and postweaning diet, with a non-significant trend for a maternal diet effect (Table 2). None of the 2-way or 3-way interactions were significant, and adding sex and cage to the model as covariates did not affect the results.

[Table 2 here]

Figures 7A-7C shows the effect of the diet switch on global microbiome composition in non-metric multidimensional scaling (NMDS) plots. Postweaning diet was a major determinant of pre-switch gut microbiota composition, with groups CC and CafC clustered away from groups CafCaf and CCaf (Figure 7A), and significant differences between groups CC and CCaf (*p*=0.001) and groups CafC and CafCaf (*p*=0.007). From pre- to post-switch there were significant within-group changes in microbiota composition in the two groups switched from Caf to chow (Figure 7B; *p*s = 0.001 and 0.023 for groups CCaf and CafCaf, respectively) but not the two groups continued on chow (Figure 7C; *p*s = .091 and .334 for groups CC and CafC, respectively). Despite the changes in the switched groups, at the post-switch timepoint there remained significant differences between groups CC and CCaf (*p*=.001) and CafC and CafCaf (*p*=.009).

A. All groups pre- and post-switch



B. Postweaning Caf groups **C. Postweaning chow groups**

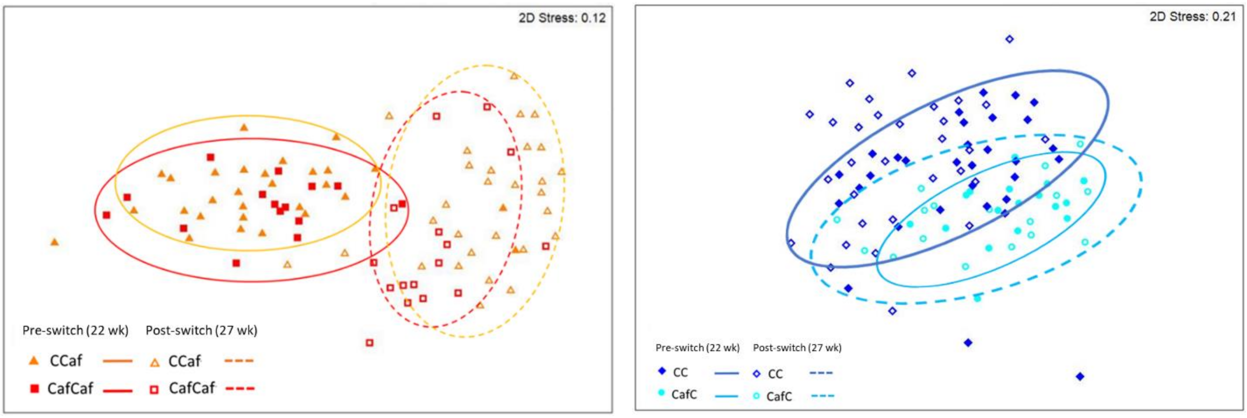


Figure 7. Gut microbiota β diversity in offspring before (Pre) and after (Post) a switch from Caf to chow diet at 22 weeks of age (CCaf and CafCaf groups) or continued access to chow (CC and CafC groups). Offspring were born to mothers fed chow or Caf diets. Non-metric multidimensional scaling (NMDS) plots following square root transformation and Bray-Curtis resemblance of relative abundance data at the OTU level. Panel A shows all offspring; Panel B shows offspring maintained on chow (CC and CafC) and Panel C shows offspring switched from Caf to chow. CC: offspring from chow mothers fed chow; CCaf: offspring from chow mothers fed Caf, then switched to chow at 22 weeks; CafC: offspring from Caf mothers fed chow; CafCaf: offspring from mothers fed Caf, then switched to chow at 22 weeks. *N*s=15-31.

Distance based linear models

The predictive relationships between host phenotype variables and gut microbiota composition pre- and post-switch were assessed using distance based linear models (DistLM) and distance-based redundancy analysis (dbRDA). Three models were tested to examine relationships between: (1) pre-switch host variables and pre-switch microbiome; (2) post-switch host variables and post-switch microbiome; and (3) post-switch host variables and pre-switch microbiome. Pre-switch place recognition memory, pre-switch percent body fat and sex were selected as predictors after assessing intercorrelations between a larger set of variables. As shown in Table 3, pre-switch gut microbiota α and β diversities were predicted by percent body fat both pre- and post-switch, with a similar, weaker trend for post-switch α and β diversity.

[Table 3 here]

Next, distance-based redundancy analysis (dbRDA) was used to explore the relationship between microbial diversity and body fat. Five common OTUs that correlated with pre- and post-switch percent body fat were *Phocaeicola*_OTU2*,* *Phocaeicola*_OTU12, *Desulfovibrio*_OTU38, *Parabacteroides*_OTU65 and *Porphyromonadaceae_unclassified*_OTU66. Figures 8A and 8B show the relative abundance of OTU2 and OTU12, respectively, from pre- to post-switch (OTU38, OTU65 and OTU66 are shown in Supplementary Figure S2). The switch to chow at 22 weeks reduced the abundance of OTU2 and OTU12 in groups CCaf and CafCaf, mirroring the reductions in body fat (see Fig. 8). In offspring of chow dams (group CCaf) abundance reduced to levels of the CC group, while OTU2 remained elevated post-switch in offspring of mothers fed Caf diet (CafCaf). BLAST searches of consensus FASTA sequences for the OTUs showed that *Phocaeicola*_OTU2 had similarity to *Bacteroides vulgatus (Phocaeicola vulgatus*) (98.8%), *Phocaeicola*_OTU12 to *Bacteroides massiliensis (Phocaeicola massiliensis*) (98.8%), *Desulfovibrio*_OTU38 to *Desulfovibrio piger* (99%) and *Parabacteroides*_OTU65 to *Parabacteroides distasonis* (97%), whereas RefSeq search identified *Porphyromonadaceae_unclassified*_OTU66 had similarity to *Muribaculum intestinale* (93.3%).

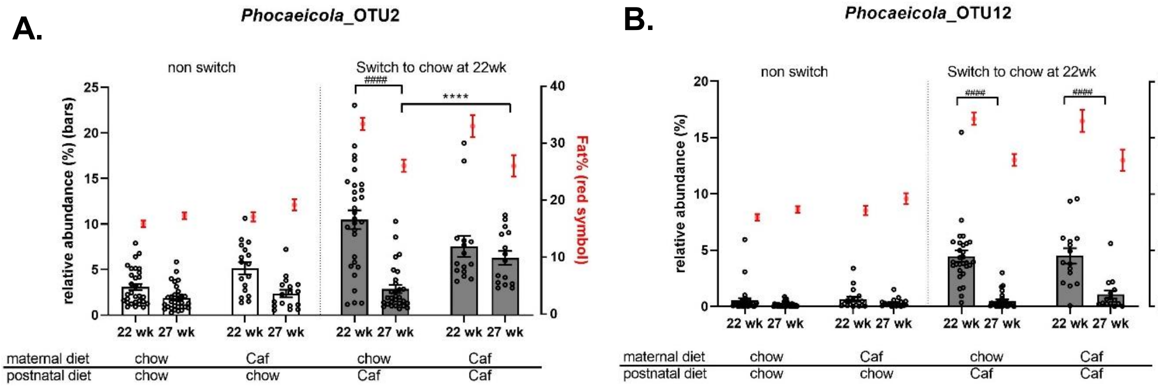


Figure 8. Relative abundance of *Phocaeicola*_OTU2 (Panel A) and *Phocaeicola*_OTU12 (Panel B), which each correlated with pre- and post-switch percent body fat. The right Y axis displays percent body fat (red symbols); the left Y axis displays OTU relative abundance (white/grey bars). Within- and between-group changes in relative abundance were analysed by Mann-Whitney and Wilcoxon tests, respectively. $^{****}p<0.0001$ Mann-Whitney test, $^{\#\#\#\#}p<0.0001$ Wilcoxon test.

4. Discussion

The present study assessed whether maternal consumption of an unhealthy diet high in fat and sugar altered the effects of switching from that diet to healthy chow in adult rat offspring. The 5-week diet switch significantly improved short-term memory, adiposity and gut microbiota species richness. The response to the switch did not differ according to maternal diet, with comparable changes in β diversity seen in Caf-fed offspring from both maternal diet groups. Adiposity reliably predicted microbiome composition before and after the diet switch. The diet switch did not fully resolve the metabolic impairments of postweaning Caf diet exposure, with body weight, adiposity and plasma leptin still elevated 5 weeks later, relative to rats fed chow, despite a dramatic and persistent suppression of energy intake. There were few sex differences in the response to the diet switch. Below we discuss the implications of these results and directions for future work.

The 5-week diet switch was implemented at ~22 weeks of age, after 19 weeks of postweaning access to chow or Caf diets. In our previous study of the younger siblings of these rats [29], maternal Caf diet exposure nearly doubled the rate of body weight gain and adiposity prior to mating, and increased body weight gain and fat mass in male but not female offspring. Here, the pre-switch measures that were collected after a further 8 weeks of diet indicated no statistically significant effects of maternal diet on offspring phenotype. Thus, even though Caf diet exposure induced a robust obesity phenotype in dams that altered offspring metabolic parameters early in life, the additional exposure to Caf diet postweaning may have overwhelmed any effects of maternal diet on metabolic parameters when assessed pre-switch in the present study. Indeed, there were substantial differences in body weight, fat mass and place recognition memory in rats fed Caf diet prior to the implementation of the diet switch.

The diet switch from Caf to chow substantially suppressed energy intake relative to groups continued on chow. Intake by the switched groups dropped rapidly by 75% relative to pre-switch levels, and was approximately half that of chow-fed controls in the first 24-h post-switch, similar to our previous work [23, 33]. Despite increasing gradually over time, intake remained significantly less than counterparts weaned onto chow even 4 weeks after the switch. The persistent suppression of energy intake in the switched groups may explain their significantly lower blood glucose at kill – an initially surprising result in light of the elevated body weight and adiposity. Such a substantial and persistent reduction in energy intake following the switch, observed in prior studies ([23], [34], [35]) may relate to the high palatability of the Cafeteria diet used, or to time spent on the diet prior to the switch. These possibilities could be tested, respectively, by replicating this design with a less palatable purified high-fat diet, and manipulating time on the unhealthy diet prior to switching to chow (see [36]).

In line with the reduction in energy intake, switching from Caf to chow steadily reduced body weight and fat mass. The extent of fat loss did not vary between offspring born to mothers fed chow or Caf diet, but was greater in Caf-fed females than males, in line with their higher proportional fat mass. Plasma insulin and triglyceride concentrations did not differ between groups at endpoint, and any differences may have washed out across the 5-week exposure to chow. Consistent with this suggestion, beneficial metabolic effects have been reported after shorter diet switches of 7-9 days [21-22]. Fat mass loss across the 5-weeks post-switch was not modulated by maternal diet. Studying more acute metabolic changes induced by the switch will be of interest, in light of recent work showing that gene expression changes in liver and adipose tissue induced by fasting were altered in offspring born to maternal cafeteria-diet fed dams [37]. While not statistically significant, it is noteworthy that in male offspring weaned onto Caf diet, terminal measures of adiposity (fat mass and leptin) were 10-20% greater in those born to mothers fed Caf, suggesting subtle effects of maternal cafeteria diet.

Of note, the switch also corrected the impairment in place recognition memory observed prior to the switch, with no statistically significant effects of maternal diet. While previous work found that maternal obesity-associated changes in gut microbiota had sex-specific effects on offspring behaviour [38], in the present study maternal diet did not interact with sex. Unlike body weight and adiposity, which remained significantly higher in postweaning Caf groups at endpoint, the switch completely removed the place recognition impairment observed in Caf-fed rats prior to the switch. This recovery agrees with a previous report where impaired contextual fear memory induced by 20 weeks access to a high-fat diet was corrected by a 4-week switch to regular chow [39]. Shorter diet switches from Caf to chow (e.g., 1 week) have been found to partially reduce the anxiolytic effects of cafeteria diet exposure [22]. However, a recent study of female rats fed Caf diet in adolescence reported that memory impairments persisted despite a 5-week switch that restored adiposity to control levels [27], unlike the present study, where adiposity remained elevated. These results suggest that the impact of a diet switch on adiposity is a function of preceding time on the unhealthy diet. Future studies can evaluate the neural correlates of recovery from cognitive impairment, which may include reductions in neuroinflammation induced by the high-fat, high-sugar diet.

An interesting possibility is that the improvement in place recognition produced by the diet switch was due to weight loss-induced reductions in leptin and consequent effects on leptin signalling in the hippocampus, where it mediates synaptic function to influence cognition [40-41]. Indeed, our previous work found that rats switched from Caf to chow exhibited a substantial increase in hypothalamic leptin receptor mRNA expression, above that of rats continued on Caf diet [23]. However, in the present study the diet switch completely restored place recognition memory despite fat mass remaining significantly elevated, suggesting that other factors may contribute to recovery from diet-induced cognitive impairment.

Our results provide tentative evidence that maternal diet altered the response of the offspring gut microbiome to the diet switch. Switching rats from Caf to chow diet for 5 weeks significantly increased α diversity species richness and Shannon diversity, but only in those born to mothers fed chow. By contrast, offspring of Caf-fed mothers that were weaned onto Caf did not show significant changes in richness or Shannon diversity in response to the diet switch. While this suggests that maternal Caf diet prevented improvements in α diversity produced by the switch, this suggestion is based on analyses of within-group changes, and the critical 3-way interaction between maternal diet, postweaning diet and switch was not statistically significant. Moreover, the failure to detect significant changes in the two groups of offspring from mothers fed Caf diet may be due to the lower sample sizes in these groups (CafC and CafCaf), relative to groups from chow-fed mothers. The diet switch substantially altered gut microbiome β diversity, with significant changes in groups switched from Caf to chow. Nonetheless, a lingering effect of postweaning diet was observed post-switch, despite the diet switch, agreeing with a recent study in young female rodents [27]. As for other measures, in the present study no effects of maternal obesity were detected on β diversity, aside from a trend toward a main effect prior to the switch.

Our predictive modelling showed that whole body adiposity was a strong predictor of pre-switch α and β diversity. Distance-based linear models indicated strong associations between gut microbiome composition and adiposity, suggesting that microbiome changes may serve as an early predictor of the metabolic response to a change in diet. Surprisingly, however, post-switch microbiome composition was less strongly associated with the phenotypic measures of interest at that time (see Analysis 2, Table 3). This may reflect distinct temporal metabolic and microbial responses to the switch, and that a stronger predictive relationship may be generated by a longer diet switch intervention.

Finally, our analyses identified several bacteria associated with the metabolic response to the diet switch. *Phocaeicola*_OTU2 (98% similarity to *Phocaeicola vulgatus*) was strongly associated with fat mass, and Postweaning Caf diet increased the abundance of this bacterium. A similar trend was observed for *Phocaeicola*_OTU12, *Desulfovibrio*_OTU38, *Parabacteroides*_OTU65 and *Porphyromonadaceae*_unclassified_OTU66. Notably, maternal Caf diet appeared to prevent a depletion of OTU2 in group CafCaf after the switch to chow, in contrast to the reduction in abundance of this bacterium in group CCaf post-switch. We previously found *P. vulgatus* to be increased in rats fed a purified high-fat diet [42] and Caf diet-fed rat dams [43], confirming reports in non-human primates fed a western-style diet [44]. A recent study in mice found sex- and tissue-dependent effects of maternal obesity on white and brown adipose tissue [45], raising the interesting possibility that *P. vulgatus* may contribute to differentiation of white and/or brown adipose tissue in offspring of obese dams. As the current study used fat mass measured by EchoMRI, we are unable to discriminate different types of fat, thus investigating the links between this bacterium and the ontogeny of fat deposition appears an important future direction. More broadly, a limitation of this study is the absence of more regular faecal and metabolic measures across the 5-week switch.

In summary, the present study suggests that an unhealthy high-fat, high-sugar diet in mothers did not alter the ability of offspring to respond to a healthy diet intervention. The diet switch led to pronounced effects on energy intake, fat mass and the gut microbiota. A diet-induced impairment in place recognition memory was restored by switching to a healthy chow diet. Importantly, on no measure were these effects different for rats born to mothers fed a healthy or unhealthy diet. It will be important in future work to identify the time-course of these changes and clarify the potential of the microbial response to the diet switch to serve as a marker for subsequent metabolic and behavioural changes.

Author contributions

Data collection: MDK AT. Data analysis and interpretation: MDK KH MJM NOK. Project conception: MJM RFW. Manuscript drafting: MDK AT KH. Manuscript revision: all authors.

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Conflict of interest statement

All authors declare no conflicts of interest.

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Table 1. Summary of PERMANOVA analyses of gut microbiota α diversity

Alpha diversity measure:	Species richness		Evenness		Shannon index	
Effect	Pseudo-F	p value	Pseudo-F	p value	Pseudo-F	p value
Maternal diet x Postweaning diet x switch interaction	$F_{(1,141)}$ =3.43	0.126	$F_{(1,141)}$ =3.07	0.143	$F_{(1,141)}$ =3.62	0.107
Maternal diet x Postweaning diet interaction	$F_{(1,141)}$=0.14	0.751	$F_{(1,141)}$=0.004	0.958	$F_{(1,141)}$=0.007	0.939
Postweaning diet x switch interaction	$F_{(1,141)}$=0.94	0.935	$F_{(1,141)}$=1.91	0.206	$F_{(1,141)}$=0.889	0.391
Maternal diet main effect	$F_{(1,141)}$=0.72	0.473	$F_{(1,141)}$=1.31	0.297	$F_{(1,141)}$=1.21	0.349
Postweaning diet main effect	$F_{(1,141)}$=20.65	**0.001**	$F_{(1,141)}$=1.321	0.291	$F_{(1,141)}$=7.30	**0.028**
Diet switch main effect	$F_{(1,141)}$=21.87	**0.001**	$F_{(1,141)}$=0.175	0.678	$F_{(1,141)}$=1.27	0.282

α diversity indices were assessed by PERMANOVA (999 permutations) using offspring gut microbiota at pre- (22 weeks) and post-switch (27 weeks). Sex was included as a covariate.

Table 2. Summary of PERMANOVA analyses of gut microbiota β diversity

Effect	Pseudo-F	p value
Maternal diet x Postweaning diet x Diet switch interaction	$F_{(1,184)} = 1.502$	$p = 0.211$
Maternal diet x Postweaning diet interaction	$F_{(1,184)} = 1.282$	$p = 0.287$
Maternal diet main effect	$F_{(1,184)} = 2.026$	$p = 0.065$
Postweaning diet main effect	$F_{(1,184)} = 8.245$	$\boldsymbol{p = 0.001}$
Diet switch main effect	$F_{(1,184)} = 6.276$	$\boldsymbol{p = 0.001}$

PERMANOVA was performed based on Bray-Curtis similarity resemblance matrix with 999 permutations. Sex/cage was nested within Postweaning diet.

Table 3. Distance-based linear modelling of pre- and post-switch microbiota and host variables.

Analysis 1: Pre-switch microbiota versus pre-switch phenotype				
	α diversity Richness	α diversity Evenness	α diversity Shannon index	β diversity
Variable	Pseudo-F	Pseudo-F	Pseudo-F	Pseudo-F
Pre-switch fat%	25.85**	39.06**	29.28**	12.99**
Pre-switch place recognition memory	3.42	4.48*	3.78	2.22
Sex	0.69	0.08	0.44	1.52
Analysis 2: Post-switch microbiota versus post-switch phenotype				
	α diversity Richness	α diversity Evenness	α diversity Shannon index	β diversity
Variable	Pseudo-F	Pseudo-F	Pseudo-F	Pseudo-F
Post-switch fat%	1.74	6.57*	2.93	2.70**
Post-switch place recognition memory	2.55	1.86	2.66	1.32*
Sex	0.24	0.90	0.39	1.76**
Analysis 3: Pre-switch microbiota versus post-switch phenotype				
	α diversity Richness	α diversity Evenness	α diversity Shannon index	β diversity
Variable	Pseudo-F	Pseudo-F	Pseudo-F	Pseudo-F
Post-switch fat%	22.4**	22.41**	18.26**	7.32**
Post-switch place recognition memory	0.12	0.12	0.10	2.22
Sex	0.08	0.08	0.44	1.52

Distance based linear models (DistLM) examined associations between α and β diversity indices and host phenotypic variables (percent body fat, place recognition memory and sex) pre- and post-switch. The table presents pseudo-F values for each variable. Statistically significant predictors of α and β diversity are indicated by *$p<0.05$ or **$p<0.01$.