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CD obese-prone rats, but not obese-resistant, robustly ferment resistant starch without increased weight or fat accretion

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Abstract

Objective—We used CD obese prone (OP) and obese resistant (OR) rats to examine how weight gain and fat accretion relate to fermentation levels and microbiota composition after feeding resistant starch (RS).

Methods—After feeding OP rats and OR rats a high fat (HF) diet for 4 weeks, rats were stratified into three groups: fed either a HF diet (HF), switched to a low fat (LF) diet or a LF diet supplemented with RS (LF-RS, 20% wt) for 4 weeks. Energy intake, body weight, fermentation variables and microbiota composition were determined.

Results—In OP rats, RS elicited robust fermentation (increased cecal contents, short chain fatty acids and serum GLP-1). Total bacteria, species of the *Bacteroidales* family *S24-7* and the archaea *Methanobrevibacter smithii* increased. The robust fermentation did not elicit higher weight or fat accretion compared to control rats fed the same isocaloric diets (HFLF±RS). In OR rats, body weight and fat accretion were also not different between HFLF±RS diets, but RS elicited minimal changes in fermentation and microbiota composition.

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Conclusions—Robust fermentation did not contribute to greater weight. Fermentation levels and changes in microbiota composition in response to dietary RS differ by obese phenotype.

Keywords

Animal Models; Dietary Fibre; Microbiome

Introduction

Gut microbiota composition affects energy balance [1–4]. Individuals predisposed to obesity may have microbial species that promote more fermentation than lean for more efficient extraction from diet [1, 2]. Studies show fermentation of resistant starch (RS) reduces abdominal fat accretion in rodents fed isocaloric diets [5–9] as RS fermentation increased fat oxidation without affecting physical activity [5].

We used outbred colonies of rats: CD obese-resistant (OR) and CD obese-prone (OP) (Charles River Company, Houston, TX). They differ in respect to their disposition to develop obesity on high fat diet [10] with both having a fully functioning leptin receptor [10–12]. Their polygenic pattern of inheritance is similar to most human obese phenotypes [11]. We investigated effects of diet on obesity phenotype and relation to gut microbiota. Belobrajdic et al. [13] reported Sprague Dawley rats fed high fat (HF) diets for 4 weeks resulted in OP and OR rats with reduced body fat when fed RS at different doses. Their study did not use isocaloric diets with RS compared to control group as diets were matched for carbohydrate content. They observed effects of diet energy dilution and fermentation of RS on weight gain. In the current study, our main comparisons had isocaloric diets in two phases of the study and focused on the effects of fermentation by gut bacteria without confounding effects of energy dilution.

We measured *Methanobrevibacter smithii (M. smithii)*, which constitutes up to 10% of anaerobic gut microorganisms [14]. A mutual relationship exists between *M. smithii* and bacterial species that ferment carbohydrates to produce H_2 and formate. Hydrogen accumulation reduces fermentation rate by inhibiting bacterial NADH dehydrogenases and ATP yield [14,15]. By consuming hydrogen in methane production, *M. smithii* improves fermentation efficacy [14]. Although this function suggests a role for methanogens in greater caloric harvest and weight gain, role of methanogens in the pathogenesis of obesity is unclear. Since fermentation is beneficial to host health [16], higher levels of *M. smithii* may be beneficial so we sought to determine how their abundance relates to the obese phenotype.

Materials and methods

Animals

The rat study was approved by the LSU IACUC. Three- and four-week-old male OP (N=32) and OR (N=32) (Charles River Laboratory) rats arrived and after one-week quarantine were singly housed in a temperature controlled vivarium with 12:12-h light/dark cycle. Rats began the study at five- and six-weeks old.

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Study plan

Our basic design (Figure 1 and Table 1) was to feed either HF or LF diet to OP and OR rats for 4 weeks (phase 1). In phase 1, 10 OP and 10 OR rats were fed LF, and 22 OP and 22 OR rats were fed HF. At end of phase 1, 4 OP and 4 OR rats were euthanized from both diet groups. For phase 2, 6 OP and 6 OR rats continued on LF for 4 weeks. The remaining 18 OP and 18 OR rats from HF in phase 1 were placed into 3 groups for each rat type. One group continued on HF, a second was switched to LF, and the third was switched to LF with 20% by diet weight as RS.

Our hypothesis, based on a previous study in which HF reduced fermentation of RS compared to LF when HF and RS were fed at the same time [7], was that HF would promote dysbiosis in rats and may affect subsequent bacterial types with feeding RS with LF. Thus, the need for a control group also fed HF in phase 1 and switched to LF without RS in phase 2. Without effect of dietary energy dilution for this comparison, OP rats may not respond as well as in Belobrajdic et al. [13] study. For phase 2, we thought OP rats may have greater accretion of abdominal fat because of greater energy harvest not offset by dietary energy dilution.

Diets and feeding

The composition of the modified AIN-93M diets are in Table 2. Weights and food intake were determined twice a week. Food and water were provided *ad libitum.* After one-week quarantine on chow there was one week acclimation period with LF diet. Rats were then fasted (~6 hr) to determine serum glucose and insulin after retro-orbital bleeding. The homeostatic model of assessment-insulin resistance (HOMA-IR) index was calculated [17]. Rats were stratified based on weight and HOMA-IR.

Serum and GI tract collections and analyses

At euthanasia, abdominal fat pads, epididymal, perirenal, and retroperitoneal, were excised. Serum was collected by heart puncture. Weight of GI tract without contents was added to the disemboweled body weight for emboweled body weight (EBW). Cecal contents were frozen in liquid nitrogen and stored at −80°C. Fermentation (cecum weights, cecal contents pH and short chain fatty acids, and serum GLP-1 active) was measured as described [7, 18–19].

Bacterial DNA extraction

DNA was extracted from 200 mg cecal contents using QIAamp DNA Fast Stool kit (Qiagen, Valencia, CA) according to manufacturer's protocol with slight modifications. In third step, zirconium beads (200 mg) and InhibitEX buffer were added and mixture subjected to bead beating (FastPrep®-24, MP Biomedicals, Santa Ana, CA) for 1 min at 6.5 m/sec (2 times). After centrifugation (20°C, 20,000 g), 500 ul of supernatant was collected. Purified DNA was quantified by NanoDrop spectrophotometry and stored at −80°C.

Primer selection

Primers (16S rRNA) for *M. smithii* were from Dridi et al. [20] and produced by Integrated DNA Technologies- (Coralville, IA) were F:5′CCGGGTATCTAATCCGGTTC3′ and R:

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5′CTCCCAGGGTAGAGGTGAAA3′. Primers for total bacteria (16S rRNA gene) were F: 5′ACGTCRTCCMCNCCTTCCTC 3′ and R: 5′GTGSTGCAYGGYYGTCGTCA3′ reported by Belenguer et al. [21]. BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed 06/22/2017) was used to verify *in silico* that primers for *M. smithii ATCC 35061* were specific (100% identity with 123 base pair double-stranded amplicon) to this species and 3 others (*M. Ruminantium*, *M. millerae*, and *M. oralis* in genus *Methanobrevibacter* and *Methanosphaera stadtmanae DSM 3091)*. Dridi et al. [20] found M. smithii in human stool samples, but also found *Methanosphaera stadtmanae* in some samples with a different set of primers. They sequenced the amplicon for their primers for *M. smithii* and found 99–100% similarity to *M. smithii ATCC 35061* so we concluded our results with these primers reflect *M.smithii*. *In silico* results for universal 16S rRNA gene matched a broad range of bacteria.

PCR amplification and amplicon DNA standards

Using Qiagen *Taq* PCR master mix kit (Qiagen, Valencia, CA), genomic DNA from pooled samples from each treatment in phase 1 was used as template to prepare amplicon DNA with specific gene sequences and lengths (123 bp *M. smithii* and 147 bp universal 16S rRNA genes). Amplicon DNA size was verified by 2% agarose gel electrophoresis. Briefly, 100 ul reactions containing 5 ul (0.5 uM) each of the forward and reverse primers, 1000 ng of template DNA and 50 ul of the PCR mix were used. Cycling conditions were: initial denaturation at 94° for 3 min, 35 cycles of denaturation at 94°C for 1 min, annealing for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10 min. Annealing temperatures were 52°C for *M. smithii* and 60°C for 16S rRNA gene. After completion of PCR, DNA amounts increased by 314 ng and 1218 ng for *M. smithii* and universal primers, respectively. These amounts were used in the formula below to calculate amplicon amounts for standard curves. Post-PCR DNA was cleaned by QIAquick PCR purification kit (Qiagen, Valencia, CA), and quantified by NanoDrop spectrophotometer. Molar concentrations of amplicon standard DNA were converted into gene copies/ul using the formula shown by Oldham and Duncan [22] assuming average molecular mass of dsDNA bp is 6.6×10^{11} ng/mol and the Avogadro's number of copies per mole is 6.022×10^{23}.

$$\text{Thus, copies per ul} = \frac{\text{DNA concentration (ng per ul)} \times 6.02 \times 10^{23}\ \text{(copies per mol)}}{\text{Amplicon length (bp)} \times 6.6 \times 10^{11}\,\text{ng/mol}}$$

Standard curves ranging from 10^8–10^2 copies/ul were generated by serial 10-fold dilutions of amplicon DNA with nuclease free water.

Quantitative Real-time PCR

The SYBR® Green qPCR assay was used to quantify total bacteria and *M. smithii* using ABI Prism 7900HT Sequence Detection System (Life Technologies, Foster City, CA). Cycling conditions were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 seconds, followed by primer annealing at 52°C for *M. smithii* and 60°C for the 16S rRNA gene, then 78°C for 30 seconds. No-template controls and amplicon standards were included in each plate. Amplicon quantities (gene copies/ul) vs. cycles to threshold (Cts) standard curves were used to determine *M. smithii* and total bacteria quantities.

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Microbiota analysis by next generation sequencing using Illumina Mi-Seq

Amplification, sequencing and bioinformatics of bacterial DNA from phase 2 were performed at the LSU Microbial Genomics Resource Group using V3 2X300 bp kits as described previously [19, 23–24]. Analysis using the UPARSE process was with 97% identity for Operational Taxonomic Units (OTUs) [19, 24].

Statistical analyses

Phase 1 data were analyzed as 2X2 factorial (OP and OR) and (LF and HF). Phase 2 data were analyzed as a 2X4 factorial (OP and OR) and (LF-LF, HF-HF, HF-LF and HF-LFRS). We used MIXED procedure of SAS 9.4 (SAS Institute, Cary, NC). Main and interactive effects were considered significant at $P<0.05$ and expressed as means ± SE. Further *a priori* comparisons used alpha level divided by number of comparisons. Next-generation sequencing data were analyzed by linear discriminant analysis (LDA) as screening tool for differences in relative abundance of OTUs among treatments (https://huttenhower.sph.harvard.edu/galaxy/). The Benjamini-Hochberg procedure [25] was applied to P values for dependent variables to decrease false discovery.

Results

Fermentation

After phase 1, no differences in weight of full and empty ceca were observed. In phase 2, OP rats fermented RS better than OR rats ($P<0.0001$, Figure 2A and 2D) as there were increases in weights of full and empty ceca of rats fed RS (diet $P<0.001$, Figure 2B and 2E). Interaction of diet and phenotype was significant ($P<0.001$, Figure 2C and 2F). There was a 65fold increase of full cecum weight and 25fold increase in empty cecum weight for OPHF-LFRS vs. OPLF-LF and OPHF-LF groups. In OR rats, the cecum weights in rats in HF-LFRS group were 30% larger than for rats fed HF-LF ($P<0.0167$).

Amounts of short-chain fatty acids (SCFAs) in cecal contents and cecal contents pH were not different after phase 1 and pH values were above pH 7 (Table S1). In phase 2, there were significant phenotype, diet and interaction effects that reflected greater amounts of fermentation in OP rats fed HF-LFRS (Table 3). *A priori* comparisons of groups with diets containing RS vs. no RS resulted in higher amounts of SCFAs (may also reflect absorption/utilization) and lower pH of cecal contents.

GLP-1 active concentrations were not different at the end of phase 1 (Table S1). After phase 2, there were increased values for GLP-1 active for LFRS diet ($P<0.0083$) (Table 3).

Energy intake, body weight and fat accretion

Energy intake in OP rats was higher than in OR rats in both phases (Figure S1). Most weight gain occurred in phase 1 (Figure 3). After phase 1, EBW was significantly higher ($P<0.0065$) in OP rats compared to OR rats while *a priori* diet comparisons showed no significant effects within rat type ($P=0.64$). After phase 2, OP rats had higher EBW ($P<0.0001$) than OR rats. Diet also had an effect and OP rats fed HF had highest increase in EBW, as there was a diet effect ($P<0.008$). The interaction of diet and phenotype was not

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significant (P=0.43) indicating relationship of weight gain among diets with two phenotypes was similar. Among *a priori* comparisons, OPHF-LFRS group had greater EBW than ORHF-LFRS group (P<0.0125) (Figure 3A). Percent increase in EBW during phase 2 was higher in OP rats compared to OR rats (P<0.005) and diet effect observed (P<0.03) because OP rats fed the HF gained the most weight. Among *a priori* comparisons, percent increase in weight in rats switched from a HF to LFRS was not different from those switched to LF without RS (Figure 3B). There was an effect of both phenotype (P<0.001) and diet (P<0.001) on total abdominal fat after phase 2. Interaction was not significant (P=0.064). Total abdominal fat and percent abdominal fat in rats switched from HF to LFRS were not different from those switched to LF (Figure 3C and 3D). Although feeding RS to OP rats increased fermentation compared to other groups, it did not contribute to greater energy storage.

Total bacteria and *Methanobrevibacter smithii* levels

In phase 1, OR rats had more total bacteria compared to OP rats (P<0.014), but diet (P=0.86) and interaction (P=0.69) were not significant. In phase 2 (Figure 4A–C), RS increased total bacteria more than 3-fold (P< 0.005) only in OP rats fed LFRS. Dietary manipulation did not change total bacteria in OR rats.

Diet and phenotype had no effect on abundance of *M. smithii* in phase 1 and HF did not change *M. smithii* levels in either OP or OR rats. In phase 2 (Figure 4D–F), there was a diet effect (P<0.02) because *M. smithii* abundance with greater fermentation increased in LFRS group by 3-fold in OP rats (P<0.005, LFRS vs. other 3 groups). There was interaction between diet and phenotype (P<0.007) because there was not a similar increase in *M. smithii* in OR rats fed RS.

Proportions of bacteria taxa

Most changes in bacterial composition occurred in OP rats fed RS (Figures 5, S2–S4). Dietary RS increased bacteria in phylum *Bacteroidetes* and reduced bacteria in *Firmicutes* in OP rats compared to OR rats (Figures S2–S4). The abundance of *Bacteroidales* family *S24-7* was highest in OP rats fed RS (P<0.001), while *Firmicutes* families *Lactobacillaceae* and *Lachnospiraceae* were more abundant in OR rats fed RS (LDA effect size, figure S4, P<0.05, Figures 5, S3–S4).

Discussion

Based on prior findings that RS fermentation reduces accretion of abdominal fat [5–9], we hypothesized OR rats would retain a lean phenotype with HF diet followed by LFRS diet compared to HFLF diets by a mechanism that involves fermentation of dietary components despite a presumably higher energy harvest with RS. Our results rejected this because OR rats exhibited modest fermentation of RS and OP rats robustly fermented RS.

The obesity trait in CD rats mimics characteristics of human obese phenotype, where not all individuals who consume increased calories develop obesity [11]; and allows delineating effects of microbiota composition on obesity phenotype and fermentation of RS. The finding that the OP rats gain greater weight and fat even on LF had not been shown before (Figure

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3B, D). Previous studies have shown they gain more weight on HF diet [10,11]. In phase 2, diet components had no effect on body weight and had a minimal effect on fermentation in OR rats. In OP rats, RS elicited very high fermentation as demonstrated by fermentation variables measured (Figure 2 and Table 2). High fermentation presumably ensures more energy harvest and has been proposed to be one of the causes of obesity [1, 2]. Studies using germ-free and knockout mice have indicated that the extracted energy is stored in adipocytes through a pathway that involves microbial down-regulation of intestinal epithelial expression of fasting-induced adipocyte factor (Fiaf), a circulating inhibitor of lipoprotein lipase (LPL). Suppression of Fiaf increases LPL activity from adipocytes, and enhances storage of liver-derived triglycerides in fat cells [1]. In the current study, dramatically increased fermentation in OP rats did not result in higher energy storage because switching rats to a LF diet or a LF diet with RS did not lead to significant differences in weight and fat accretion in OP rats (Figure 3). The absence of robust fermentation in OR rats fed RS was unexpected, given that this type of rat strain is reported to remain insulin sensitive and maintains leaner phenotype when fed increased calories [10–11]. Neither OP nor OR rats responded to fermentation with decreased body fat accretion. Our past study observed increased fatty acid oxidation [5] and this may be the result of stimulation by increased SCFAs reaching liver via the portal blood [26] causing reduced accretion of abdominal fat in several rat models fed RS [5–9]. The results with OP and OR rats may be explained by their development from breeding of heavier Sprague Dawley rats (Charles River Company). However, it is surprising that both OR and OP models were developed. OR and OP rats may harvest enough energy from fermentation to balance any increase in fat oxidation and do not reduce fat accretion or have a reduced metabolic response to fermentation. It is also possible that feeding RS without prior feeding of HF, might have produced a different result.

Our study had different results than Belobrajdic et al. [13] possibly because we focused on the effects of fermentation with isocaloric diets for our comparisons of HF-LFRS groups (OP or OR) to HF-LF groups rather than combined effects of fermentation and dilution of energy density. With resistance to digestion, RS has a lower metabolizable energy than control starches [27]. Belobrajdic et al. [13] used RS to replace control starches, and RS diets in their study had lower amounts of energy. Secondly, our study used commercially established CD-OP and CD-OR rat strains, but Belobrajdic et al. [13] used Sprague Dawley rats. We discovered an OR rat model that is a low-fermenter of RS. Such a model could serve as a model for humans that are reported to be low-fermenters [28–30].

Amounts of total bacteria and the archaeon *M. smithii* determined by qPCR were not different between the HF and LF diets (phase 1). OR rats had significantly higher amounts of total bacteria than OP rats, but the amount of *M. smithii* was not significantly different. Including RS in the diet (phase 2) increased both total bacteria and *M. smithii* in OP rats, but not in OR rats (Figure 4). In absence of dietary RS, the role of methanogenic archaea and particularly *M. smithii* in the pathogenesis of obesity is not clear. Mathur et al. [15, 31] showed that methanogenic archaea contribute to altered metabolism and weight gain in the host. They observed higher proportions of *M. smithii* in humans with obesity compared to lean counterparts and genetically obese mice when compared to lean littermates. Gut colonization with *M. smithii* correlated with and predicted the degree of weight gain [15]. In contrast, our results showed that *M. smithii* levels were not affected by the obese phenotype

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but were affected by fermentation of RS. Furthermore, the increase in *M. smithii* levels was beneficial to the host as it appeared to contribute to enhanced fermentation with no extra weight gain or fat accretion. Mathur et al. [15] and Mathur and Barlow [31] did not elaborate on components of diet, particularly on amounts of fermentable fiber. The observed higher proportions of *M. smithii* in humans with obesity and genetically obese mice may have been errantly attributed to the obese phenotype rather than to greater amounts of fermentable fiber.

Another bacterial composition increase in OP rats in response to RS was in abundance of *S24–7*, an uncultured gram-negative family of order *Bacteroidales* (Figure 5). *S24–7* is involved in host-microbe interactions that impact gut function and health and abundance is altered with different conditions [32] and in the current study in response to feeding of RS. Analysis of 16S rRNA gene databases from metagenomics by Ormerod et al. [32] showed that members of the family *S24-7* are fermentative but have alternative modes of energy production as they encode elements of an electron transport chain. Like other families in *Bacteroidales* order, carbohydrate-active enzymes constitute about 6% of *S24-7* coding sequences. Based on enzyme abundance, *S24-7* encode glycoside hydrolases; largely α-amylases suggesting starch as key substrate, with ability to ferment several other carbohydrate moieties [32].

We conclude that increases in *M. smithii* and *S24-7* are markers of bacterial fermentation of RS because both rat phenotypes had similar amounts of these potential markers in phase 1 when no RS was fed. We previously observed increased *S24-7* in obese ZDF rats that robustly fermented RS [19], and hypothesize we would have observed an increase in *M. smithii* if these were measured in other studies. Increases in *S24-7* and *M. smithii* may be under a homeostatic mechanism of host dynamics that regulates the gut community responses to promote host fermentation. The robust fermentation in OP rats did not elicit reduced food intake and did not result in extra weight or fat accretion, which demonstrated that fermentation does not appear to be the cause of obesity in OP rats. OR rats may be a good model for humans reported to be low fermenters of RS and the cause of their low response to RS is unknown. It may be related to their microbiota and inability to increase *M. smithii* and *S24-7* levels. In OP rats, host environment favored RS fermentation and *M. smithii* and *S24-7* were then beneficial to the host for increased fermentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Study Importance Questions

1. What is already known about this subject?

- CD obese-prone rats gain more weight and fat than CD obese-resistant rats when fed a high-calorie diet.

2. What does your study add?

- Robust fermentation of dietary resistant starch in CD obese-prone rats does not increase body weight and abdominal fat accretion.
- CD obese-resistant rats poorly ferment dietary resistant starch and may serve as a model for humans that poorly ferment resistant starch.
- Archaeon *M. smithii* amounts were similar in CD obese-prone and obese-resistant rats when not fed resistant starch, but increased with robust fermentation in CD obese-prone rats.

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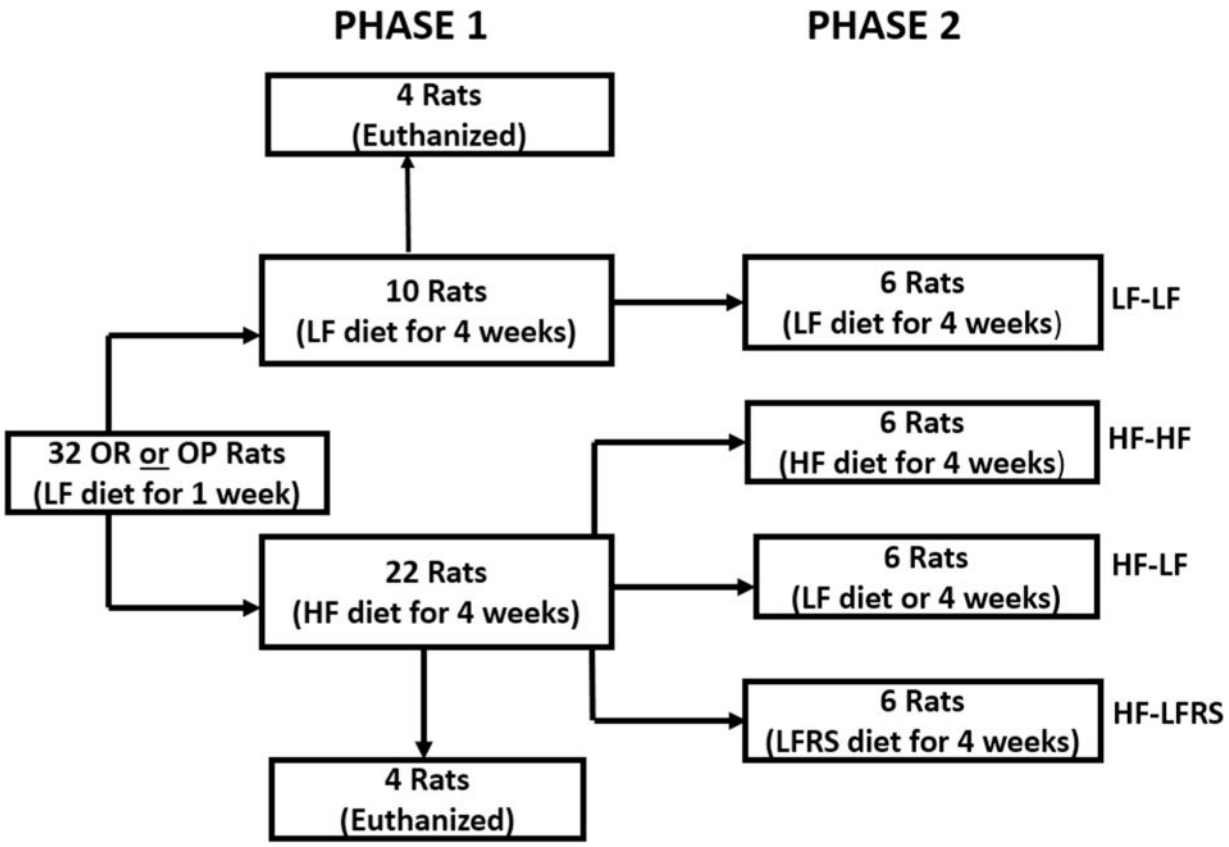

Figure 1.
The study design showing the study with two phases with both OP and OR rats.

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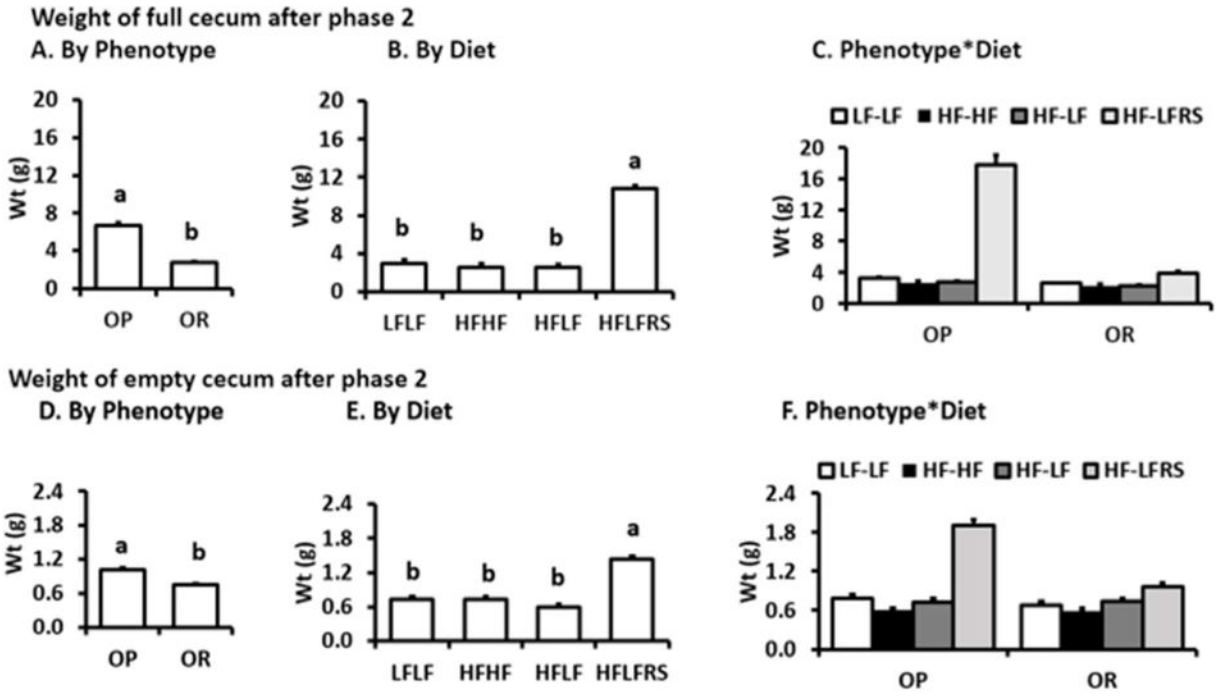

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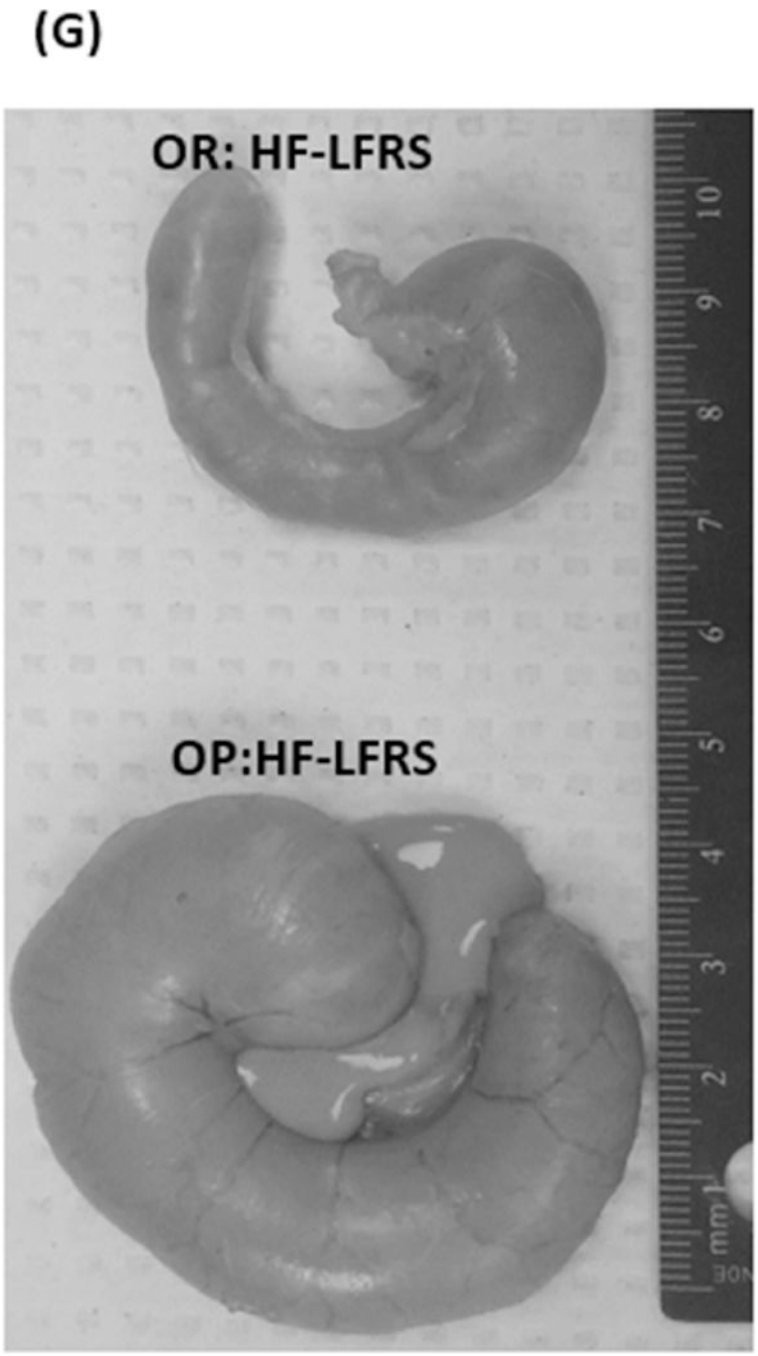

Figure 2. Weight and size of ceca

Full ceca (A–C) and empty ceca (D–F). In A and D, the letters designate differences between OP and OR rats ($P<0.0001$). For B and E the letters denote *a priori* differences between HF-LFRS and the three other diets ($P<0.0167$). For panels C and F there were differences ($P<0.0125$) between *a priori* comparisons: OPHF-LFRS vs OPLF-LF, OPHF-LFRS vs. OPHF-LF, OPHF-LFRS vs. ORHF-LFRS and ORHF-LFRS vs. ORHF-LF. G shows a representative picture of ceca of OP and OR rats fed RS.

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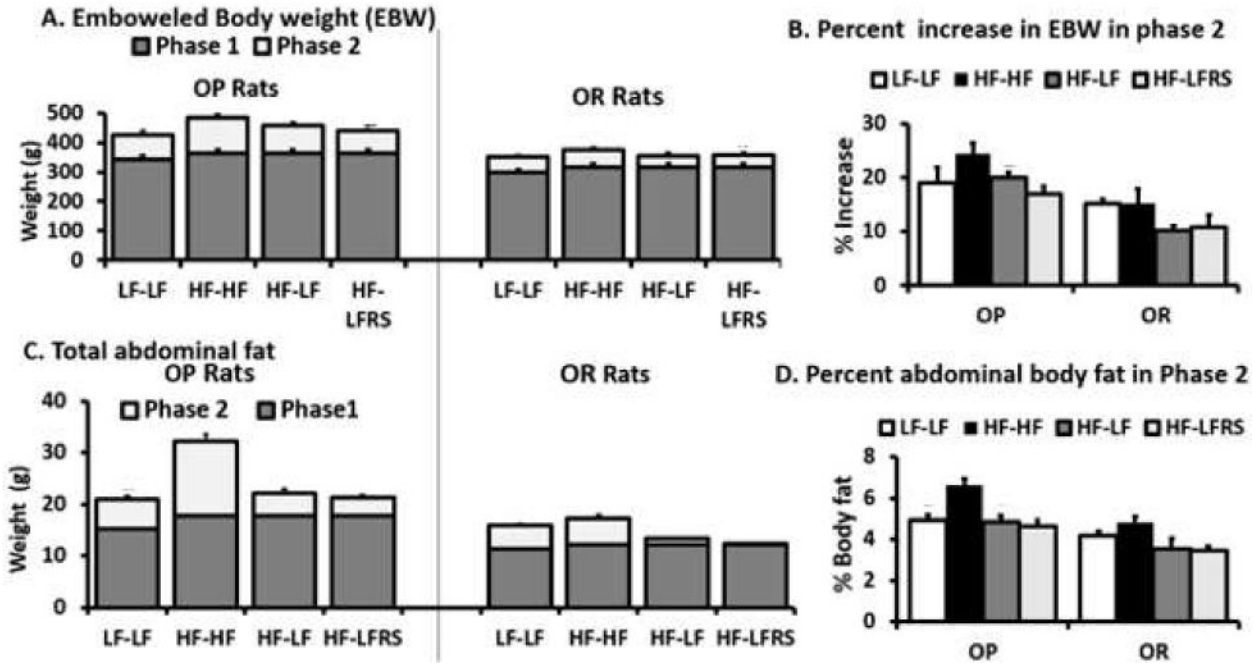

Figure 3. Body weight and body fat

A. Weight of the GI tract contents was subtracted from total body weight to determine emboweled body weight (EBW) for OP and OR rats. B. Percent increases in EBW as phase 2/phase 1*100. C. Total abdominal fat. D. Percent abdominal fat (abdominal fat/EBW*100) after phase 2. In A and C data for the lower darker bars was determined from the 4 animals per LF and HF diet groups euthanized after phase 1. Data for the upper lighter bars was determined from the 6 animals per group after phase 2. The only *a priori* difference after phase 2 for A-D was between OPHF-LFRS vs. ORHF-LFRS ($P<0.0125$). There were no significant differences for OPHF-LFRS vs OPHF-LF, OPHF-LFRS vs OPLF-LF and ORHF-LFRS vs ORHF-LF.

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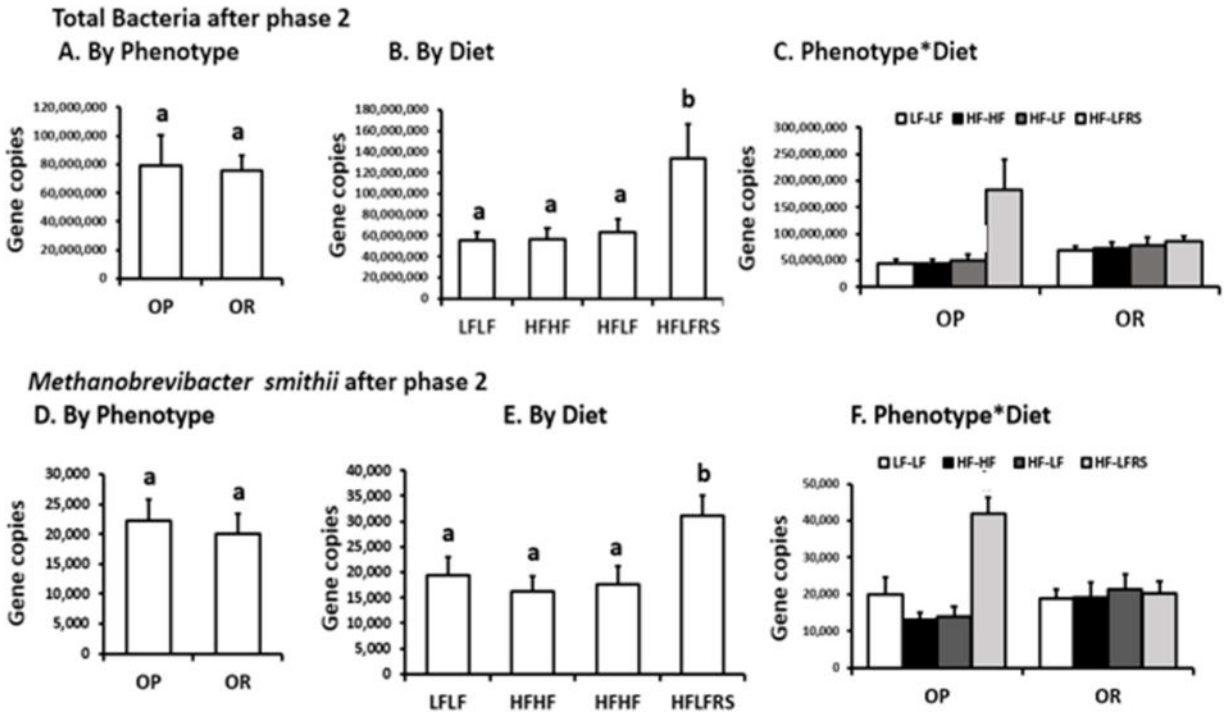

Figure 4. Abundance of total bacteria and *Methanobrevibacter smithii* after phase 2 (qPCR)

A–C. Total bacteria. D-F. *M. smithii*. For A and D, there were no significant differences in both total bacteria and *M. smithii* for OP and OR rats. The letters a and b in B and E indicate three *a priori* differences P<0.0167) between HF-LFRS diet and all other diets. In C and F for OP rats there were *a priori* differences (P<0.0125) between OPHF-LFRS vs. OPHF-LF, ORHF-LFRS vs. OPLF-LF, and OPHF-LFRS vs. ORHF-LFRS. No significant difference was observed between ORHF-LFRS and ORHF-LF.

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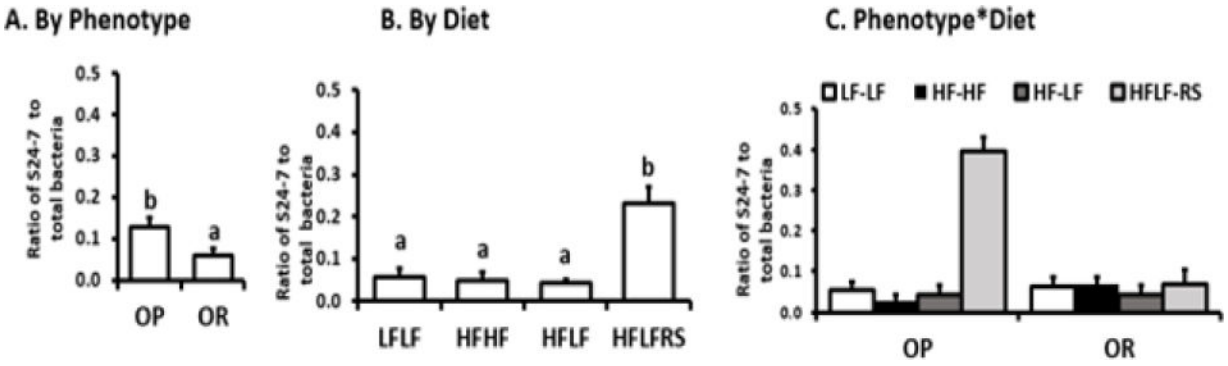

Figure 5. Relative abundance of the family S24–7 gene copies (qPCR) data after phase 2 (S24-7/ total bacteria)

A. OP rats had higher abundance of S24–7 compared to OR rats (P<0.003) indicated by different letters. B. The HF-LFRS diet had increased *S24-7* abundance compared to all other diets (P<0.0005). The letters a and b indicate *a priori* differences between OPHF-LFRS and OPLF-LF, OPHF-LF and ORHF-LFRS (P<0.0125) C. Interaction between diet and phenotype influenced *S24-7* abundance (P<0.0001). There were *a priori* differences (P<0.0125) between OPHF-LFRS vs. OPLF-LF, ORHF-LFRS vs. OPLF-LF, and OPHF-LFRS vs. ORHF-LFRS. No difference was observed between ORHF-LFRS and ORHF-LF.

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Table 1

Rat and Diet Acronyms for Phases 1 and 2 of Study

Acronym	Description
OP	CD obese-prone rat (Charles River Company)
OR	CD obese-resistant rat (Charles River Company)
LF	Low-fat diet
HF	High-fat diet
LF-LF	Low-fat diet in phases 1 and 2 of study
HF-HF	High-fat diet in phases 1 and 2 of study
HF-LF	High-fat diet in phase 1 with switch to low-fat diet for phase 2
HF-LFRS	High-fat diet in phase 1 with switch to low-fat diet with 20% resistant starch by weight in phase 2

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Table 2

Diets for CD obese-prone and obese-resistant rats

Diet components		**Low fat diet**		**High fat diet**
Ingredients (g)	***Energy value Kcal/g***	**LF (g)**	**LF-RS (g)**	**HF (g)**
[1] 100% amylopectin cornstarch	3.5	521.1	147.1	405.7
[2] HM260 (containing RS at 20% of diet[3])	2.8	0	472.4	0
Sucrose	4.0	100	100	100
Casein	3.50	140	140	140
Cellulose	0	150.8	52.4	106.2
Corn oil	8.84	40	40	100
Lard	9.00	0	0	100
Mineral mix (AIN -93M)	0.88	35	35	35
Vitamin mix (AIN 93)	3.87	10	10	10
Choline chloride	0	1.3	1.3	1.3
L-Cystine	4	1.8	1.8	1.8
Total weight (Kcal)		1000 g (3160.2)	1000 g (3160.1)	1000 g (4182.5)

[1] 100% amylopectin cornstarch is the AMIOCA® cornstarch product from Ingredion Incorporated (Bridgewater, NJ).

[2] HM260 is high-amylose cornstarch (HI-MAIZE® 260 resistant starch) from Ingredion Incorporated (Bridgewater, NJ).

[3] HM260 lot was 42.3% RS based on wet weight as used in diet.

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Table 3

Fermentation variables: SCFAs, pH of cecal contents and GLP-1 active in serum[1]

Variable	Diet	OP	OR	P value (Phenotype)	P value (Diet)	P value (Phenotype*Diet)	Pooled SEM
Acetate (mmol/cecum)	LF-LF	0.081	0.045	<0.0001	<0.0001	<0.0001	0.007
	HF-HF	0.056	0.038				
	HF-LF	0.061	0.032				
	HF-LFRS	0.401	0.095				
Propionate (mmol/cecum)	LF-LF	0.015	0.009	<0.001	<0.001	<0.0001	0.001
	HF-HF	0.010	0.006				
	HF-LF	0.030	0.004				
	HF-LFRS	0.049	0.008				
Butyrate (mmol/cecum)	LF-LF	0.020	0.010	<0.001	<0.001	<0.0001	0.0017
	HF-HF	0.014	0.008				
	HF-LF	0.016	0.007				
	HF-LFRS	0.127	0.022				
pH	LF-LF	7.46	7.62	<0.001	<0.001	<0.0001	0.973
	HF-HF	7.56	7.57				
	HF-LF	7.56	7.30				
	HF-LFRS	5.62	6.90				
GLP-1 (pmol/L)	LF-LF	0.97	0.89	0.20	0.0083	0.839	0.073
	HF-HF	1.15	1.13				
	HF-LF	0.88	0.99				
	HF-LFRS	1.66	1.06				

[1]For all Table 2 variables there were differences (P<0.0125) for four *a priori* comparisons (OPHF-LFRS vs. OPLF-LF, OPHF-LFRS vs. OPHF-LF, OPHF-LFRS vs. ORHF-LFRS and ORHF-LFRS vs. ORHF-LF).