RESEARCH ARTICLE

Molecular Nutrition & Food Research
www.mnf-journal.com

# Early-Life Sugar Consumption Affects the Microbiome in Juvenile Mice

*Reem Moath Alasmar, Kavitha Varadharajan, Muralitharan Shanmugakonar, and Hamda A. Al-Naemi**

**Scope: The composition of the gut microbiota is influenced by the dietary nutrient. Sugar has been linked with many metabolic health disorders such as heart disease, metabolic syndrome, and immune disorders. Long-term consumption of sugar influences the landscape of gut microbiota by altering the gut microbial population called dysbiosis. This study aims to evaluate the impact of long-term consumption of high sugar diet (HSD) on the diversity of gut microbiota.**

**Methods and results: CD1 mice are given high concentration of sugar for 15 weeks followed by a recovery period of 10 weeks. Real-time polymerase chain reaction and 16S rRNA next-generation sequencing methods employ to identify microbiome diversity. The results show that *Firmicutes* and *Bacteroidetes* are the predominant phyla in control, cecum, and fecal samples. *Firmicutes* population are gradually increased in treated samples even after the recovery period, whereas *Bacteroidetes* abundance slightly reduces throughout the study.**

**Conclusion: The present study shows that the impact of long period of high sugar diet consumption alters the diversity of normal gut flora which can be restored after 10 weeks of sugar withdrawal. This indicates that the intervention of healthy and nutritious diet influences gut microbes and this can be beneficial in reducing the implication of early life metabolic disorders such as obesity.**

## 1. Introduction

Obesity is a global public health problem and the prevalence of obesity is increasing aggressively leading to high rate of morbidity and mortality. Lifestyle changes and accessibility to high fat and high sugar fast food with sedentary lifestyle fuels the epidemic of obesity worldwide.[1] However, attempts to study the longitudinal effects of diet-induced body weight gain in the human population challenged by ethical and traditional considerations. Rodent animal models such as rats and mice play a major role in studying obesity due to their close identity to humans in terms of genetics and physiology. Thus, rodent obese models are an attractive analogy for human obesity studies. In addition, obesity harms human health in many different ways; the effective management of this problem needs a comprehensive understanding of both the pathogenesis and cellular physiology.[2] Evidence suggests that gut microbiota plays an essential role in metabolic, nutritional, physiological, and immunological processes.[3] Therefore, different diet shapes the microbiome population, and their landscape in the gut maintained in a balance with different microbiota.

The excess energy intake from high sugar diet (HSD) considered as the main cause of metabolic disorders leads to changes in gut microbiota and this has been studied in detail using obese mouse models.[1] The link between energy imbalance and metabolic disorders such as obesity and diabetes is supported by research data collected from human and animal studies. Humans consume a high amount of sugars most likely in the form of sucrose.[4] Sucrose is a disaccharide composed of an equal amount of monosaccharides in the form of glucose and fructose. Monosaccharides are the main sources of energy for living organisms.[5] However, glucose is the most predominant circulating monosaccharide used as a source of energy that is linked with metabolic diseases.[6] Many research studies illustrated the correlation between glucose homeostasis, obesity, insulin resistant, and diabetes.[7] However, fructose hemostasis, gut microbiomes, and possible contribution in obesity and diabetes needs more investigation especially towards understanding

R. M. Alasmar, K. Varadharajan, M. Shanmugakonar, H. A. Al-Naemi
Laboratory Animal Research Centre
Qatar University
Doha, Qatar
E-mail: halnaemi@qu.edu.qa
H. A. Al-Naemi
Department of Biological and Environmental Sciences
Qatar University
Doha, Qatar

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202200322



**DOI: 10.1002/mnfr.202200322**

the possible link between high consumption of HSD in early-life, and microbiome diversity.[6] Once the carbohydrates get digested by the digestive system, digested monosaccharides molecules are absorbed and transported across the cell membrane by facilitated diffusion through membrane protein known as glucose transporter (GLUT).[5] Monosaccharides are mainly transported by sodium-glucose cotransporter (SGLT1) and facilitative Unitrans porter GLUT2 and GLUT5.[8–10] During high glucose levels, facilitated diffusion through GLUT2 takes place to transport extra glucose load. In the small intestine, the rate of fructose absorption is slower than glucose.[11] Therefore, long-term consumption of high concentrations of fructose results in availability of excessive amounts of fructose that exceed receptor transporting capacity and leads to incomplete absorption, fluid retention, and fructose malabsorption.[12] Poorly absorbed fructose molecules move to the large intestine where it fermented by intestinal microflora called "Gut microbiota." The human gut hosts about 39 trillion microorganisms that contribute over 100 times more genes than the human genome.[6] Microbial inhabitants of the gut enriched with various types of microorganisms and live in harmony by maintaining a balance among themselves called "Gut microbiota." In general, the predominant microbiota classes are gram-positive *Firmicutes* and gram-negative *Bacteroidetes*.[13] The recent data form our lab showed that the microbiota can be sub-divided into different enterotypes, each enriched by unique bacterial genera, but that all seem to have a high degree of functional uniformity, they are re-subdivide again depending on a long period of high sugar diet intake.[14] Gut microbiota dysbiosis due to high-fructose diet intake causes epithelial barrier dysfunction by increasing intestinal permeability or leaky gut.[15] Also, imbalance in gut microbiota have been linked with chronic gastrointestinal conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), and diseases with broader systemic manifestations such as type 2 diabetes, obesity, and atopy.[16] However, the exact causes of these diseases are unknown and is thought to be multifactorial. Genetic factors, environmental factors, GIT dysfunction, infection, inflammation, and immunity disorder are thought to play roles in developments.[13] Together with these factors inflammatory bowel disease and irritable bowel syndrome tends to have less bacterial diversity and lower numbers of *Bacteroidetes* and *Firmicutes* ratio is thought to be low-grade intestinal inflammation.[16] Obesity and type 2 diabetes are complex disorders affected by both genetic and environmental factors.[17,18] Further results indicate that the ratio of *Firmicutes* to *Bacteroidetes* in the gut of obese mice was shifted in favor *Firmicutes* whereas the gut of the control mice was dominated by *Bacteroidetes*.[17] Many reports explained that the structure of gut microbiota is influenced by diet-induced changes in their population, and the impact of their dysbiosis in cellular events.[14,17] However, to our knowledge, very few studies focused on exploring the impact of a chronic high sugar diet intake on early-life gut microbiota. There are limited studies on how the dysbiosis in gut microbiota could be re-populated with a normal healthy microbiome when the high-sugar diet withdrawn and replaced by normal diet. In the present study, we hypothesize that changes in the taxonomical abundance of gut microbiota, their distribution, and localized changes in the cecum and large intestine occur when juvenile mice are fed for long period of time with chronic high-sugar diet.

## 2. Experimental Section

### 2.1. Animal Care and Husbandry

Outbred CD-1 Mice were obtained from the breeding colony at the Laboratory Animal Research Center (LARC), Qatar University (QU), Qatar. The animals have been housed in individually ventilated cages (IVC) under standard animal husbandry conditions (temperature—20–24 °C; humidity—30–70%; 12 h light/12 h dark cycles). All animals maintained on normal chow diet (carbohydrate 56.9%; protein 18.0%, and lipids 4.8%) and water ad libitum. All experimental protocols were approved by Qatar University Institutional Animal Care and Use Committee (QU-IACUC 4-3/2019-AMM1) and Institutional Biosafety Committee (QU-IBC-2019/014).

### 2.2. Experimental Design

Seventy Juvenile (4 weeks old) CD-1 male mice (bodyweight 12 g ± 0.1) were randomly assigned as Control (C, $n = 32$) group and high sugar diet (HSD) treated group (HSD, $n = 38$). The control animals received normal chow diet and normal drinking water, whereas HSD treated group received normal chow diet and 30% sugar water (commercially available table sugar obtained from the local market) for 15 weeks, then the HSD treated animals reversed to normal drinking water as a recovery period for 10 weeks (**Figure 1**). During the 25 weeks of the study, both groups had accessed to a chow diet and drinking water ad libitum. Weekly fed and water consumption and body weight gain recorded accordingly.

### 2.3. Sample Collection, Processing, and DNA Extraction

Animals were euthanized by $CO_2$ standard method, cecum fecal and fecal samples were collected under aseptic conditions from all experimental groups at weeks 15 and 25 of the study. DNA extracted from fecal pellets and cecum fecal samples using QIAamp DNA Stool Mini kit (Qiagen). From each experimental group, fecal pellets and the cecum fecal samples were weighed (≈180–220 mg) and homogenized individually with InhibitEX buffer provided in the kit and followed the extraction procedure according to the kit protocol. The quantity and quality of the extracted DNA analyzed using an IMPLEN nanophotometer and stored at −80 °C for further analysis.

### 2.4. Real-Time PCR Analysis

Analysis of gut microbiota diversity was conducted using a Quantstudio 6 Flex Real-Time PCR system (Applied Biosystem) with SYBR green master mix with specific primers. In order to identify the target 16s rRNA gene of the gut microbiota (*Firmicutes* and *Bacteroidetes*), primer sequences were used.[13] To verify the specificity of the amplicon, agarose gel electrophoresis was performed in addition to a melting curve analysis. Based on the relative intensity of amplicons captured by the gel documentation system (Syngene) and the microbial abundance ratio calculated by Image J analysis software.[13]

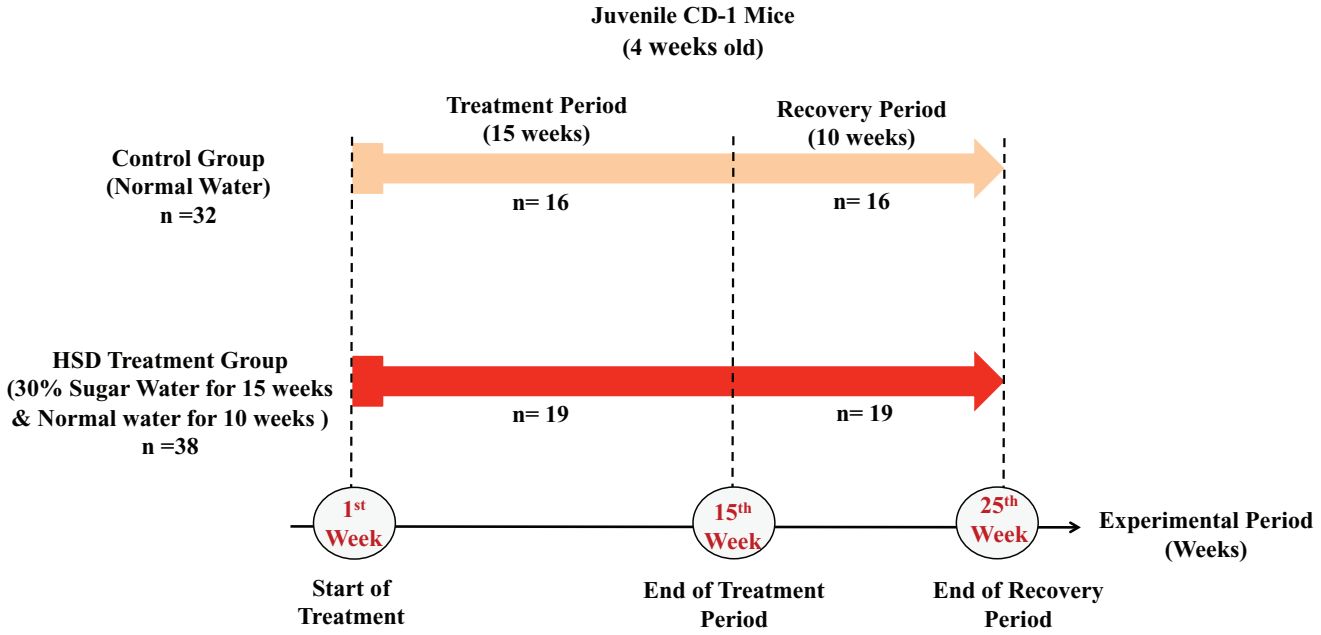


**Figure 1.** Outline of the high sugar diet (HSD) treatment and recovery period of the current study.

### 2.5. Next-Generation Sequencing and Bioinformatics Analysis for Diversity Analysis

Next-generation sequencing (NGS) analysis outsourced and performed by Macrogen (Korea). Extracted DNA samples subjected to quality check (QC) and the samples subjected to library preparation (Hercules II fusion DNA polymerase Nextera XT Index Kit V2) followed by 16S metagenomics analysis performed using Illumina sequencing platform (MiSeq System). After sequencing, the raw data were filtered using Quantitative insights into microbial ecology (QIIME) software, which was capable of filtering multiplexed sequence reads and operable taxonomic units (OTUs). A clustered and aligned sequence dataset used to identify gut microbiota taxa. The assembled-OTU analysis tables used to report the relative abundance of the microbiota in the representative experimental sample. The diversity of the microbial population was explored using the QIIME package.[14]

### 2.6. Statistical Analysis

The data represented as the mean ± standard error of the mean. The statistical significance of the differences among groups was determined by one-way ANOVA analysis to compare the mean between the groups (Control and Treated) with Tukey's analysis using GraphPad Prism software. One-way ANOVA test was accomplished and the differences were considered statistically significant when $p$-value < 0.05.

## 3. Results

### 3.1. Physiological Condition of the Mice

In this study, 4-week-old male CD-1 mice fed normal chow diet and provided with normal drinking water (control) and 30% sugar water (treated) ad libitum. Feed and water intake of the experimental animals monitored and recorded throughout the study. Due to the palatability of sugar water, water intake is significantly higher in HSD treated groups compared to control groups received normal drinking water in the course of treatment period (15 weeks). However, consumption of drinking water reduced irrespective of groups in the recovery period (after withdrawn sugar water) in the treatment groups (Figure 1). With respect to feed intake, control animals consumed marginally more than the HSD treated animals in the treatment period whereas HSD treated groups consumed more than the control groups in the recovery period. Both control and HSD treated groups gained weight in the initial experimental period. Consistently, body weight monitoring showed that the mice with free access to sugar water started to gain significantly more body weight than the mice with normal drinking water and this trend observed for the rest of the experiment. At the end of the experiment, the body weight of the HSD group was higher than the control group and the animal maintain their body weight during recovery period. High sugar-diet consumption results in body weight gain on HSD treated group 30% of them are physiologically obese.

### 3.2. Gut Microbiome Analysis Using Real-Time PCR

A high sugar diet is likely to be the major factor responsible for alteration in similarity and structure of the gut microbial composition. **Figure 2** explains the abundance ratio of major phyla *Firmicutes* and *Bacteroidetes* along with important beneficial and non-beneficial bacteria in the cecum and fecal samples that were influenced by HSD. Concerning gut microbial abundance ratio in cecum, when compared to control samples *Firmicutes* population gradually increased (15–24%) in HSD treated samples even after recovery period increment in abundance observed

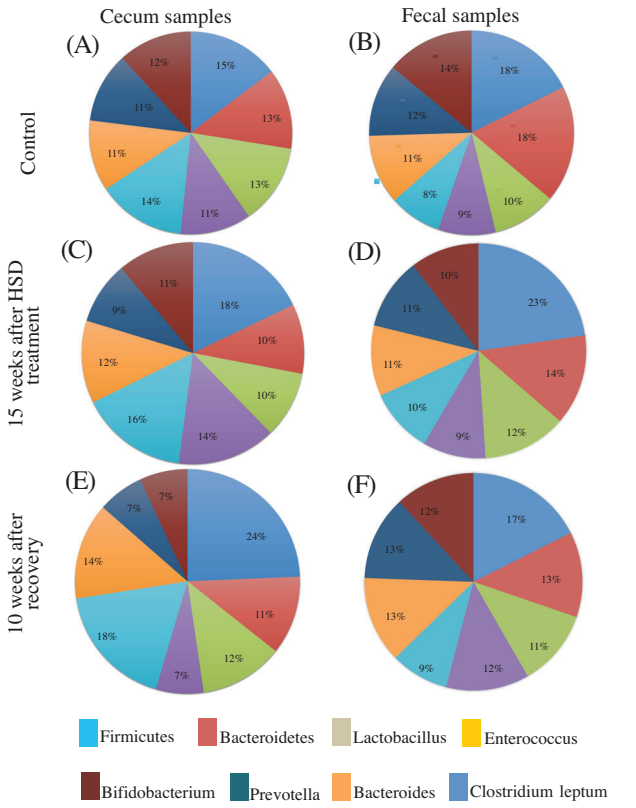


**Figure 2.** Explains the ratio (%) distribution of gut microbiota population after high sugar diet (HSD) treated and control groups of the cecum and fecal samples on treatment (15 weeks) and recovery period (10 weeks) by Real-Time PCR analysis.

whereas, *Bacteroidetes* abundance slightly reduced and maintained throughout the study. In addition, beneficial bacteria like *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, and *Bacteroides* also studied individually, and HSD alters the abundance ratio in different scenarios. *Lactobacillus* gradually reduced during the HSD treatment period and restored after week 25. *Enterococcus* showed inconsistent behavior in both treatment and recovery periods. There are significant alterations in the *Bifidobacterium* ratio (12%) on control samples and it gradually reduced to (11%) on the treatment period and (7%) after the recovery period whereas *Bacteroides* gradually increased and attain 14% at the recovery period. Non-beneficial bacteria *Clostridium leptum* showed a significant increase in its abundance even after the recovery period (14–18%) whereas *Prevotella* gradually reduced to (7%).

Concerning gut microbial abundance ratio in fecal samples, when compared to control samples *Firmicutes* population gradually increased (18–23%) in HSD treated samples and significantly reduced to 17% after the recovery period whereas *Bacteroidetes* gradually decreased from 18% to 13% after the recovery period. Beneficial bacteria like *Lactobacillus*, *Enterococcus*, *Bifidobacterium*, and *Bacteroides* showed different patterns of alterations upon HSD treatment. *Lactobacillus* abundance start increased (12%) after 15 weeks of treatment and reduced (11%) after the recovery period. There is no significant change in *Enterococcus* even after 15 weeks of HSD treatment however increase in percentage after the recovery period (12%). The abundance ratio of *Bifidobacterium* gradually reduced (10%) and recovered (12%) after the recovery period. *Bacteroides* showed inconsistent behavior in both treatment and recovery periods. *C. leptum* gradually reducing (10%) after 15 weeks of treatment compared to the control sample whereas *Prevotella* showed abundance observed (13%) after recovery period (Figure 2).

Analyzing microbial community at the phylum, family, and species level indicates that there is a rich diversity of gut microbes in all samples of HSD fed animals that showed the higher abundance of 10% *Bacteroidetes*, 18% *Firmicutes* at the phylum level, and 12% *Bacteroides*, 10% *Lactobacillus*, 16% *C. leptum*, 14% *Enterococcus*, and *Prevotella* showed less than 10%. While, after 15 weeks of received HSD treatment, shifting in gut microbiota population was observed in fecal samples, the abundance of 14% *Bacteroidetes*, 23% *Firmicutes*, at phylum level and 11% *Bacteroides*, *12% Lactobacillus*, *10% C. leptum*, 11% *Prevotella* at the species level and *Enterococcus* showed >10% of their abundance. When compared to the control animals, gut microbiota varied significantly in the cecum and fecal samples. However, in the cecum sample, *Firmicutes* (24%) and *C. leptum* (18%) were more abundant when compared to *Bacteroidetes* (11%), *Bacteroides* (14%), and *Lactobacillus* (12%) and all others are less than (10%) of the population. Moreover, *Firmicutes* (17%), *Bacteroidetes*, *Bacteroides*, and *Prevotella* (13%) dominated in fecal samples of recovery animals but all others are less than (12%). In contrast, there is not that much dominance in the cecum and fecal of control groups, and the entire phylum, family, and species distributed in the range of (7–16%) and (6–25%). Moreover, *Bifidobacterium* gradually reduced (17–7%) in the treatment group as well in the recovery group in both samples (Figure 2).

### 3.3. 16s rRNA Metagenomic Sequencing (NGS) Analysis

To determine the impact of HSD on gut microbes inhabiting in the cecum and fecal samples, 16S rRNA metagenomic analysis performed to identify alterations at the phylum, class, order, family, genus, and species levels. Genomic DNA extracted from cecum fecal and fecal samples of control, treated, and recovery animals. Three replicates from each group were subjected to 16S rRNA metagenomic sequencing analysis, short-read sequencing libraries were prepared from the extracted DNA and hypervariable region V3–V4 of the 16S r DNA was sequenced, the representative results are presented in the current study. Interestingly, we found distinct changes in HSD-fed mice compared to the control and recovery animals at the phylum, class, order, family, genus, and species levels.

**Figure 3** explains the gut microbial taxonomical abundance ratio of Control, HSD treated, and recovery cecum fecal samples using 16s rRNA metagenomic analysis. In cecum samples phylum level, *Firmicutes*, *Bacteroidetes*, and *Deferribacteres* are predominant in cecum control samples whereas *Proteobacteria* and *Actinobacteria* shared a very less percentage in treated and recovery samples. The abundance ratio of *Deferribacteres* (7%) increased in HSD treated samples and reduced (2%) after the recovery period. The abundance ratio of *Firmicutes* reduced (39%) after HSD treatment and back to normal (43%) after the recovery period. There is no significant difference observed with phylum

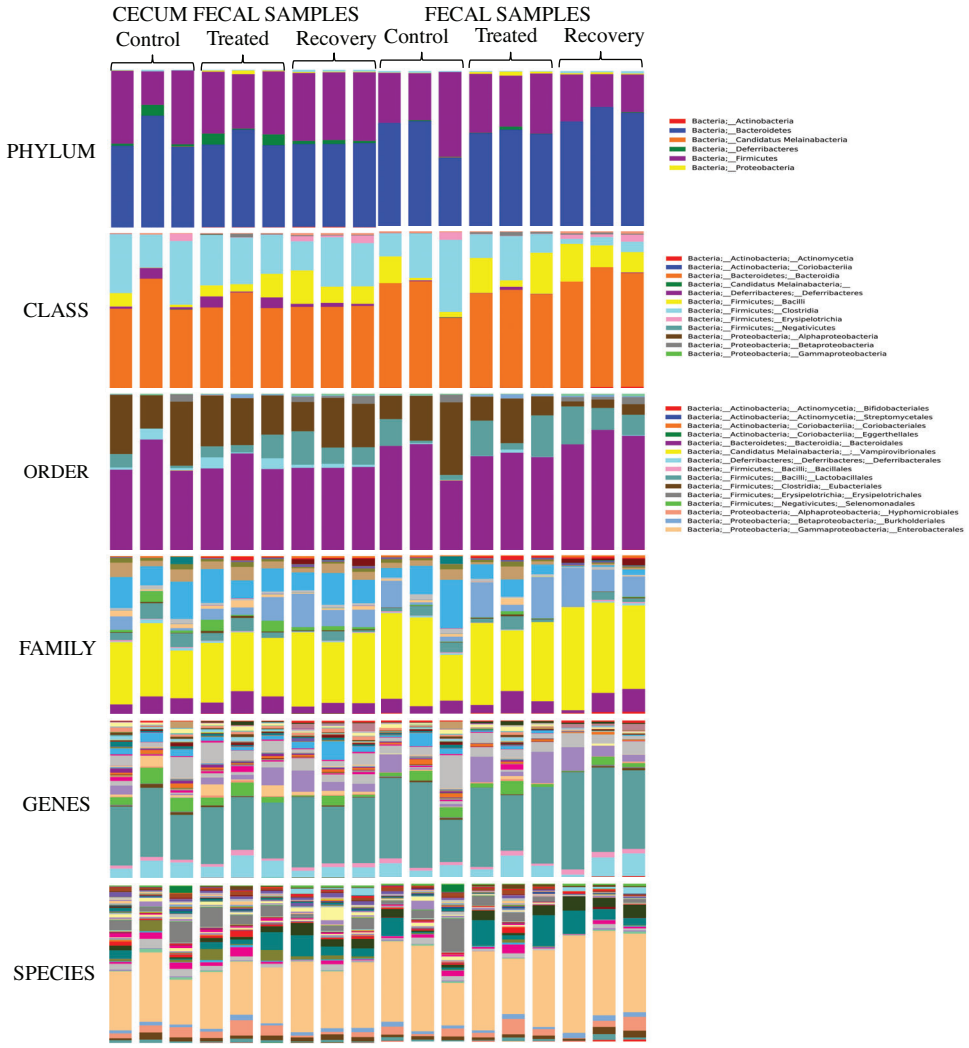


**Figure 3.** Explains the percentage of variance of NGS-derived community structure of gut microbial taxonomy of Control, HSD treated, and recovery cecum and fecal samples using 16s rRNA metagenomic analysis per individual sample. A detailed figure legend is attached in the supplementary excel file S1, Supporting Information.

*Bacteroidetes* (55.3%) irrespective of diet. At the class level, *Bacteroidia* completely ruled in the entire *Bacteroidetes* population whereas *Bacilli*, *Clostridia*, *Erysipelotrichia* from *Firmicutes* have predominated ratio. Others like *Deferribacteres* from *Deferribacteres*, *Actinomycetia* from *Actinobacteria*, *Betaproteobacteria* from *Proteobacteria* are predominant. In order level, *Lactobacillales*, *Eubacteriales*, and *Erysipelotrichales* from *Firmicutes* are high in their abundance whereas *Bacteroidales* from *Bacteroidetes*. Others like *Deferribacterales* from *Deferribacteres*, *Bifidobacteriales* from *Actinobacteria*, and *Burkholderiales* from *Proteobacteria* are contributed significantly. At the family level, *Firmicutes* dominated with *Turicibacteraceae*, *Peptococcaceae*, *Oscillospiraceae*, *Lachnospiraceae*, and *Lactobacillaceae* families whereas *Rikenellaceae*, *Muribaculaceae*, and *Bacteroidaceae* were dominated by *Bacteroidetes*. At the genes level, *Firmicutes* shared huge microbial diversity whereas *Bacteroidetes* shared *Muribaculum*, *Alistipes*, *Duncaniella*, and *Bacteroides* predominantly. At the species level, *Bacteroidetes* shared *Muribaculum intestinal*, *Alistipes putredinis*, *Duncaniella frater*, and *Bacteroides rodentium* predominantly observed.

Figure 3 explains the gut microbial taxonomical abundance ratio of control, HSD treated, and recovery fecal samples using 16s rRNA metagenomic analysis. In fecal samples the phylum level, *Firmicutes* and *Bacteroidetes* are predominant irrespective of control and treated samples whereas *Deferribacteres*, *Proteobacteria*, and *Actinobacteria* shared a very little percentage in treated and recovery samples. Compared to the control, abundance ratio of *Firmicutes* slightly increased in HSD treated samples whereas *Bacteroidetes* reduced and the abundance of *Bacteroidetes* significantly increased to more than 70% of the total population. On the *other* hand, the *Proteobacteria* ratio increased (2.5%) in treated samples and reduced (>1%) during the recovery period whereas *Actinobacteria* increased (1%) in recovery samples. Class and order levels of fecal samples are much similar to cecum samples; however, the abundance ratio varies due to the

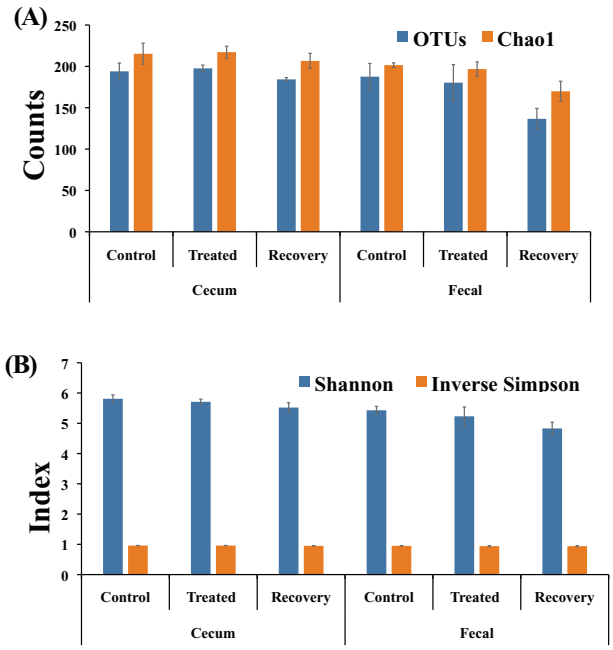


**Figure 4.** a–b) Explains microbial community richness and evenness by OTUs, Chao1, Shannon, and Inverse Simpson Index.

varied function at the different region of the gut. At the family level, *Oscillospiraceae*, *Lachnospiraceae*, and *Lactobacillaceae* families were dominated by *Firmicutes* while *Rikenellaceae*, *Muribaculaceae*, and *Bacteroidaceae* were dominated by *Bacteroidetes* this is similar to cecum samples. Genes and species of fecal samples are also very similar to cecum but the abundance ratio varied from the cecum.

Phred quality score of all the cecum and fecal samples showed high accuracy of each nucleotide. All samples expressed more than 99% and 96% accuracy of Q20% and Q30% respectively. All the experimental samples attained more than 180 operational taxonomical units (OTUs) though few samples showed more than 120 OTUs is more than sufficient for sequencing analysis. Microbial community richness and evenness analyzed by OTUs, Chao1, Shannon, and Inverse Simpson that reveals good coverage (**Figure 4**a,b). In addition, the rarefaction curve generated according to PD whole tree analysis exceeds 10 000 sequences per sample observed irrespective of all samples (**Figure 5**a,b). The rarefaction curves obtained from control, treated, and recovery animal samples with variations in the depth of the sequencing; this was used to visually demonstrate the reasonability of the amount of sequencing data. The *x*-axis of the rarefaction curves shows the amount of sequencing data extracted from samples and the *y*-axis represented the number of species. The three lines of the three groups represent gradually became flat, which means the amount of sequencing data was appropriate.

Distance matrices of the observed sequence from the cecum and fecal samples were analyzed by Principal Coordinate Analysis (PCoA) weighted Unifrac plot analysis. PCoA weighted unifrac plot defined the relative abundance of gut microbial species that was shared between the experimental samples. In 2D-PCoA, the distance matrix is explained according to control,

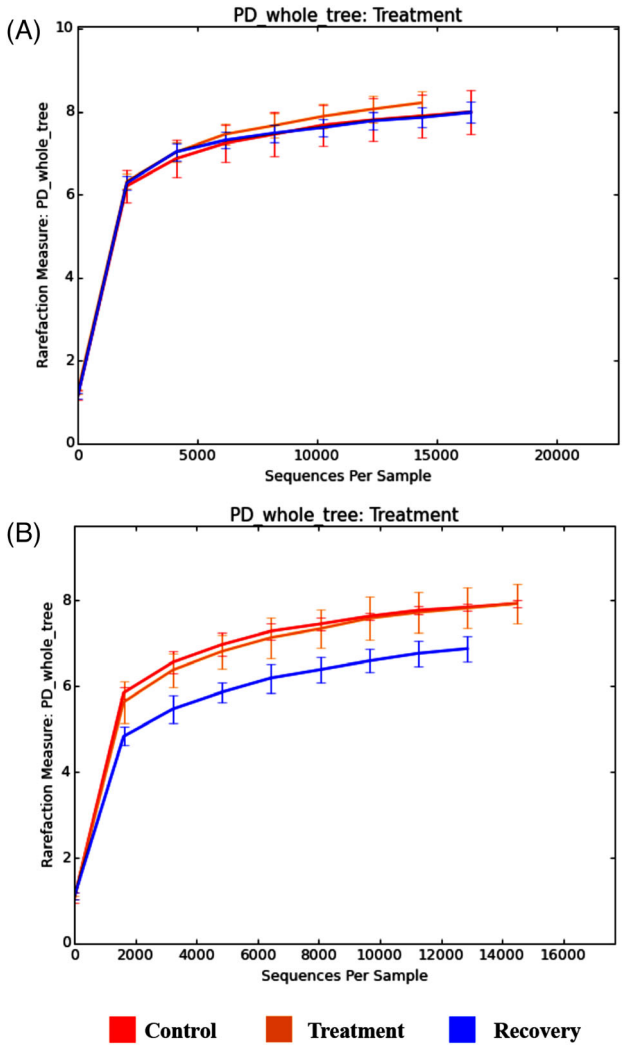


**Figure 5.** a–b) Rarefaction curve analysis of the PD-whole tree: treatment a) cecum and b) fecal samples. The lines of the three groups gradually became flat in fecal samples more than cecum samples, which indicate the quantity of sequencing data.

treated and recovery samples (Figure S1a, Supporting Information) according to the cecum and fecal samples (Figure S1b, Supporting Information). In 3D-PCoA, the plot clearly explains the gut microbial communities in the cecum fecal and fecal samples are well-distributed with 24.58% of PC1, 15.03% of PC2, and 11.56% of PC3-PCoA (**Figure 6**). Both 2D and 3D PCoA analysis reveals a clear distance matrix between control, treated, and recovery samples. The unweighted pair group method with the arithmetic mean (UPGMA) tree indicates the hierarchical clustering and distance matrix of the experimental samples (Figure S2, Supporting Information). These results indicate that diets with high sugar intake shape distinct gut microbiota in treated animals.

## 4. Discussion

The finding of this study supports our hypothesis that the high sugar diet influences the composition of the gut microbiome

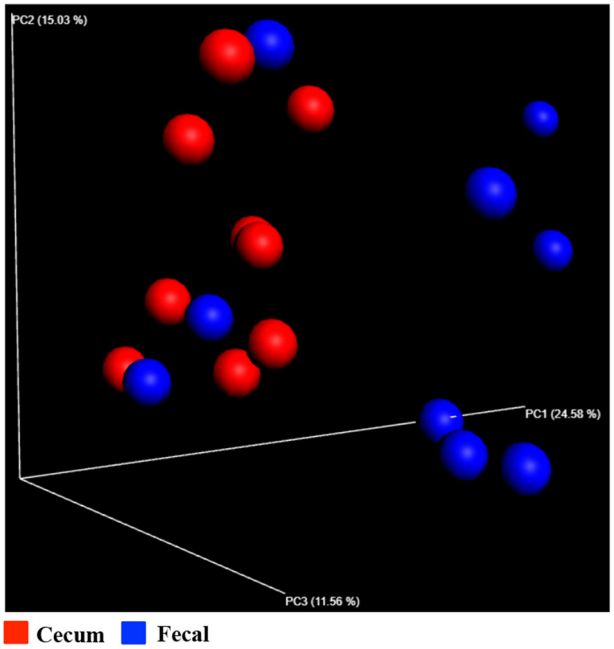


**Figure 6.** 3D-Principal coordinate analysis (PCoA) of the gut microbiome in cecum and fecal samples. Every dot indicates the bacterial population composition of individual fecal and cecum samples. Axis titles explained the percentage difference.

and increases the abundance of microbes belonging to the taxonomic group closely associated with the development of obesity. In addition, the re-appearance of essential microbes that have probiotic effect when the animals refed with normal water after 15 weeks of high sugar diet consumption. Our finding indicates that altered gut microbial flora can be restored with the intervention of healthy food and that explains the role of beneficial gut microbes towards a healthy life. Many research studies describe the effect of sugars on gut microbial physiology and their impact on the destabilizing balance between beneficial and non-beneficial gut microbiota. For example, glucose is actively absorbed in the small intestine through glucose transporters (GLUT), while only 5–30% of fructose can able to absorb in the small intestine. Nevertheless, the small intestinal gut microenvironment is enriched ≈10-fold in sugar receptors when compared with the large intestine.[14] The fructose absorption rates are slower than glucose molecules because of different absorption processes taking place in the small intestine between those two monosaccharides. All of these available sugars appear to be important substrates for microbes in the small intestine as well as in the large intestine.[19] Moreover, microbiota from the small intestine is significantly enriched with diversity of genes and enzymatic capabilities that facilitate efficient sugar absorption and utilization compared to those from large intestines.[20]

On the other hand, the selection of bacteria driven by the diet and the food available to the gut microbiota. As a result, selected bacteria can control the host feeding behavior to enrich and increase their fitness. Microbes in the gut can induce cravings for specific diets that are optimal for their growth. Several studies are showing that gut microbial products and their metabolites detected in the systemic circulation has the potential to regulate appetite and satiety directly in the hypothalamus.[21] Hence, in our study we did explore the microbial diversity in mice cecum and fecal samples of HSD fed animal groups using RT-PCR and 16s rRNA metagenomics analysis.

Studies suggest that the absence of microbiota (mice raised in germ-free environments) may protect against diet-induced obesity, but possibly not for all mouse strains and diets. Several mechanisms explain how specific microbiota composition can affect host adiposity.[22] The study done by Kobyliak et al. explained different methods such as, increased availability of short-chain fatty acids generated by the bacterial breakdown of complex polysaccharides, giving the host access to more calories and can be persuaded by inducing inflammation, which may result in insulin resistance and excessive food intake (hyperphagia).[23]

In our study, we focused on analyzing microbial community from cecum and fecal samples that harbors a rich diversity of gut microbiota.[14,24] Our findings describe the abundance ratio of gut microbiota in cecum fecal and fecal samples of control, HSD fed, and HSD recovery group. With respect to real-time PCR experiments, cecum and the fecal samples abundance ratio of *Firmicutes* are more than *Bacteroidetes* upon HSD treatment. These results are consistent with that of Renato et al. (2021), in the HSD fed group, an increase in *Firmicutes* and depletion in *Bacteroidetes* observed. While after the recovery period (Week 26), the *Firmicutes* (24%) populations in cecum samples retained their population increasing and dominant in the cecum whereas in fecal samples *Firmicutes* returned to normal (17%). In agreement with the previous study, the prevalence of phylum *Firmicutes* tend to increase in HSD groups and the *Bacteroidetes* population significantly reduced in the cecum and fecal samples in the same group.[25]

Interestingly, cecum fecal samples of treatment and recovery period shows the abundance of *Bifidobacterium* (17%) and *Prevotella* (13%) as a dominant population at the beginning of HSD diet. However, after 10 weeks of receiving HSD the population of these two bacteria start decreasing in their abundance (11%, 9%) and the trend continued (7% for both) even after stopped receiving HSD that is in the recovery period. These results along with previous reported findings demonstrate that *Bifidobacterium* and *Prevotella*, are beneficial bacteria they can produce short-chain fatty acids (SCFAs).[18] It is reported that their abundance significantly decreased after high-sugar and high-fat diet indicating a shortage in their capacity for providing energy for the gut microbial population in mice.[25] This outcome is contrary to Lee[26] stating that the *Prevotella* population is associated with a high sugar intake of carbohydrates and simple sugar. *Lactobacillus* populations in our study maintaining their abundance the same level as control groups. In addition, *Bacteroides* are potentially considered as harmful and pathogenic bacteria with inflammatory effect in the gut region of mice. Our observation on unpredictable abundance of *Bacteroides* in the cecum and fecal samples during HSD as well as recovery period is contrary to the finding of Santacruz et al.[27]; they reported a significant increase in the *Bacteroides* population when the mice received HSD. All these results indicate that HSD destabilizes the balance of the gut microbiota and activates the pathogenic gut microbiota in response to inflammation. When compared with control animal group, in cecum fecal samples, *Enterococcus* increased during the HSD period and decreased on recovery period. While in the fecal

samples, *Enterococcus* maintain their abundance same as control group during HSD treatment however increased trend observed during the recovery period. A shift in microbiota population induced by HSD at different regions of the gut observed both in HSD treatment and recovery groups; but the rate of metabolism and its pathway may vary.[28]

Taxonomical analysis through the NGS system play a major role in studying the microbiota and most of the recent studies using rodent models focused on the ratio of *Firmicutes* and *Bacteroidetes* that influence the diet-induced obesity in rats. Only few studies focused on the impact of HSD on the ratio of both *Firmicutes* and *Bacteroidetes*.[14] In our study, we observed a higher abundance of *Bacteroidetes* (55.42% and 60.33%) in the treated cecum and fecal samples compared to *Firmicutes* population due to high sugar diet (30%). *Bacteroidetes* are gram-negative and anaerobic bacteria that exist in the gut of mice and the increase in abundance of *Bacteroidetes* supports the phenomenon of diet induces obesity. Varadharajan et al.[14] reported similar results in increased abundance of *Bacteroidetes* due to a high-fat diet and high glucose in the cafeteria diet in rat model. However, in our study the relative abundance of *Actinobacteria* reduced in HSD groups and increased in recovery groups in both cecum and fecal samples.

Interestingly, the abundance of *Deferribacteres* (7.05%) altered and become more in HSD treated groups and reduced during the recovery period (2.57%) in cecum fecal samples. *Deferribacteres* reported to elevate in the gut microbiome of HSD and HFD-induced obesity models. *Deferribacteres* ferment sugar molecules to enrich their population and involve in triggering metabolic signaling pathways that can induce obesity.[29] *Deferribacteres* have also found to be a beneficial microbe especially when it is associated with a host microbiota, and it can prevent the development of new species, particularly pathogens. However, some studies have indicated that it may cause diseases. Moreover, microbiome profiling showed that *Mucispirillum schaedler*, a member of the phylum *Deferribacteres*, was associated with the cecum of mice and found in the intestinal microbiota population in humans.[30] Mice colonized with *M. schaedler* showed reduced gut inflammation after infection with *S. typhimurium*, this suggests that *M. schaedler* protects the animals from *S. typhimurium*-induced colitis.[30]

*Proteobacteria* one of the pathogenic bacteria, gram-negative present in abundance in HSD groups (2.39–2.53%) and decreased in the recovery period (0.48–0.43%) on both samples (cecum and fecal) sites and these increases may also induce the obesity in the treated animals. This trend is in agreement with our earlier observation that an increase in the abundance of *Proteobacteria* may also induce obesity in the animals fed with HFD.[14] Also, metabolic disorders that lead to obesity revealed microbiota dysbiosis are associated with an increased prevalence of *Proteobacteria*.[31] There are plethora of studies on the altered ratio of microbiota at the phylum level, however few studies focused more on the microbiota structure at the class, order, family, and genera level. In our study, we observed alterations of microbiota composition at the class level especially the *Firmicutes* are highly significant. In cecum and fecal samples, *Clostridia*, *Bacilli*, and *Erysipelotrichia* that are three classes predominant within *Firmicutes*, but only class *Bacteroidia* contributes within *Bacteroidetes* phyla. Our data agree with a study performed by Etxeberria et al.[32] on diet-induced obese mice showing bloom in the *Clostridia*, *Bacilli*, and *Erysipelotrichia* within *Firmicutes* phyla. Moreover, there are two orders predominant in *Firmicutes* phyla, although only one order is dominant in *Bacteroidetes* irrespective of sample sites. Furthermore, we also discuss the microbial diversity within the different families found in the cecum and fecal samples. The *Bacteroidetes* increased in the treatment group in both cecum and fecal samples (14.04%, 14.06%), however decreased (4.35%) in the recovery group in cecum samples. Within the Bacteroidetes phylum, *Muribaculaceae* family shows an increasing trend in both cecum and fecal samples in all the groups. As far as the phylum *Firmicutes* is concerned a remarkable increase in the ratio was observed in treated samples when compared to the recovery and control groups. In the control group, the *Lactobacillaceae* showed a more reduction than in treatment and recovery groups.

In HSD treated samples, there was no significant difference in *Lactobacillaceae*, *Eubacteriales*, and *Oscillospiraceae*. *Turicibacteraceae* whereas increased significantly in recovery animals in both cecum and fecal samples. In addition, we found a significant alteration in *Proteobacteria* phylum and suggest us that they may play a critical role in diet metabolism.[6] Since in our study the *Sutterellaceae* population increased only in treated samples while it reduced in recovery samples in both sites. This finding is in line with our previous work reported on cafeteria diet-fed rat models.[14] Rest of all families was similar to fecal samples and there are no significant differences between groups (control, treated, and recovery).

At the genus level, *Bacteroidetes* were found to distribute more abundantly than *Firmicutes*, but *Firmicutes* exhibited more variation in terms of the number of genera. Within the *Bacteroidetes*, only six different genera were observed, whereas over 30 different genera were found within *Firmicutes*. The abundances of *Allobaculum*, *Blautia*, *Ruminococcus*, *Bacteroides*, *Mucispirillum*, *Dorea*, *Anaerovorax*, *Corobacillus*, *Alistipes*, *Lactobacillus*, and *Tannerella* were significantly higher in the HSD-treated group, while *Prevotella*, *Coprococcus*, *Turicibacter*, *and Rikenella* were significantly higher in control and recovery groups. With regard to the species level, there were abundant populations of *Murobaculum intestinal* observed irrespective of groups. Each *Lactobacillus* species (*Lactobacillus intestinalis* and *Lactobacillus johnsonii*) has a different effect on weight change that is host-specific. More research needed to clarify the role of *Lactobacillus* species in regulating mice energy and body weight.

All above observations highlight that the level of diversity of gut microbiota not substantially linked with the phenotype of the animals, the site of the gut, or the diet composition. Every microbiota has its own metabolic pathway, which can change depending on various factors like diet, environmental influences, and genetic variations. The abundance of microbiota populations can also vary depending on the time of sample collection, the age of the animal, the amount of diet consumed, and the duration of metabolism. Bacterial metabolism (hormones, endotoxins, and antibiotics) can have an indirect effect on a host phenotype due to hormonal alterations. The metabolism is influenced by species variation and abundance that determines the phenotype of the animals. This study selected the Juvenile CD-1 mice fed directly after weaning (4 weeks old), because we found that early life diet source had more long-lasting effects on the gut microbiome diversity and will influence on long-term human

health and development. These findings clearly provide an idea to the public about children who starts their life with high sugar diet can develop obesity and other metabolic disorders due to the influence of sugar on development and healthy metabolic machinery. This might continue their entire life even after stop consuming sugar; moreover, not all the gut microbes can be brought back due to sugar free diet because some of the family and species might maintained their dominance in the gut. All the above results highlight gut microbiota alteration and the landscape of gut microbiota because of high sugar diet. Understanding the gut microbiota population enhance our knowledge on how this change contributes to the signaling pathways and other related immune response in our body.

## 5. Conclusion

In conclusion, we demonstrate that chronic consumption of HSD induces obesity in CD-1 mice. Diet is a major determining factor of the gut microbial dysbiosis. The response of the gut microbiota to dietary impact is often rapid; alternations observed within 4 weeks. Our study focuses mainly on the role of microbiome on host–microbial interactions, their distribution, and abundance in different regions of the gut. Profiling Cecum and fecal microbiota community by RT-PCR and next-generation sequencing provides a valuable information about their diversity, specificity, stability, and development dynamics in a short period of high sugar diet exposure. Analyzing microbial community at the phylum, class, order, family, genus, and species level indicates that there is a rich diversity of gut microbes in all samples of HSD-fed animals. Highlight of this study is defining the influence of diet on microbiota in early life stage. It is imperative to be aware of some changes that happened in the gut microbiome of obese animals and how these changes have impact on the physiological changes in order to develop effective treatment strategies to treat the metabolic disorders. However, due to the complexity of our physiological machinery further studies need to be conducted to identify and understand how the high sugar diet induced microbiota changes leads to inflammation and metabolic disorders.

## Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

## Acknowledgements

The authors would like to acknowledge LARC for their support and LARC vivarium team, for their support in animal care and husbandry. The research is funded by Qatar University.

Open Access funding is provided by the Qatar National Library.

## Conflict of Interest

The authors declare no conflict of interest.

## Author Contributions

R.M.A.: writing – original draft, methodology, formal analysis, data curation. K.V.: conceptualization, methodology, visualization, investigation, reviewing, and editing. M.S.: resources, visualization, reviewing and editing, supervision. H.A.A.: conceptualization, animal modeling, investigation, visualization, writing – reviewing and editing, supervision, project administration.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Keywords

CD1 mice, gut microbiota, high sugar diet (HSD), next-generation sequencing (NGS), real time-PCR

Received: May 17, 2022
Revised: July 31, 2022
Published online:

[1] Z. B. Wang, S. S. Xin, L. N. Ding, W. Y. Ding, Y. L. Hou, C. Q. Liu, X. D. Zhang, *Evid. Based Complement. Altern. Med.* **2019**, *2019*, 3862971.
[2] N. Zeeni, C. Daher, G. Fromentin, D. Tome, N. Darcel, C. Chaumontet, *Stress* **2013**, *16*, 211.
[3] A. M. Martin, E. W. Sun, G. B. Rogers, D. J. Keating, *Front. Physiol.* **2019**, *10*, 428.
[4] A. Tagliabue, M. Elli, *Nutr. Metab. Cardiovasc. Dis.* **2013**, *23*, 160.
[5] S. Z. Sun, M. W. Empie, *Nutr. Metab.* **2012**, *9*, 89.
[6] M. Do, E. Lee, M. J. Oh, Y. Kim, H. Y. Park, *Nutrients* **2018**, *10*, 761.
[7] H. L. Bradlow, *Horm. Mol. Biol. Clin. Investig.* **2014**, *17*, 53.
[8] C. Patel, V. Douard, S. Yu, P. Tharabenjasin, N. Gao, R. P. Ferraris, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2015**, *309*, R499.
[9] J. Beisner, A. Gonzalez-Granda, M. Basrai, A. Damms-Machado, S. Bischoff, *Nutrients* **2020**, *12*, 3444.
[10] R. D. Feinman, E. J. Fine, *Nutr. Metab. (Lond)*. **2013**, *10*, 45.
[11] R. P. Ferraris, J. Choe, C. R. Patel, *Annu. Rev. Nutr.* **2018**, *38*, 41.
[12] K. Ebert, H. Witt, *Mol. Cell. Pediatr.* **2016**, *3*, 10.
[13] R. M. Alasmar, K. Varadharajan, M. Shanmugakonar, H. A. Al-Naemi, *Adv. Microbiol.* **2019**, *09*, 775.
[14] K. Varadharajan, M. Shanmugakonar, S. C. Das, H. A. Al-Naemi, *Adv. Microbiol.* **2018**, *08*, 975.
[15] R. K. Singh, H. W. Chang, D. Yan, K. M. Lee, D. Ucmak, K. Wong, M. Abrouk, B. Farahnik, M. Nakamura, T. H. Zhu, T. Bhutani, W. Liao, *J. Transl. Med.* **2017**, *15*, 73.
[16] D. N. Frank, A. L. St. Amand, R. A. Feldman, E. C. Boedeker, N. Harpaz, N. R. Pace, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 13780.
[17] M. J. Bull, N. T. Plummer, *Integr. Med.* **2014**, *13*, 17.
[18] I. Rowland, G. Gibson, A. Heinken, K. Scott, J. Swann, I. Thiele, K. Tuohy, *Eur. J. Nutr.* **2018**, *57*, 24.
[19] S. C. Di Rienzi, R. A. Britton, *Adv. Nutr.* **2020**, *11*, 616.
[20] R. M. Alasmar, K. Varadharajan, M. Shanmugakonar, H. A. Al-Naemi, R. M. Alasmar, K. Varadharajan, M. Shanmugakonar, H. A. Al-Naemi, *Adv. Microbiol.* **2019**, *9*, 775.
[21] S. O. Fetissov, *Nat. Rev. Endocrinol.* **2017**, *13*, 11.
[22] J. Miyamoto, M. Igarashi, K. Watanabe, S. Karaki, H. Mukouyama, S. Kishino, X. Li, A. Ichimura, J. Irie, Y. Sugimoto, T. Mizutani, T. Sugawara, T. Miki, J. Ogawa, D. J. Drucker, M. Arita, H. Itoh, I. Kimura, *Nat. Commun.* **2019**, *10*, 4007.
[23] N. Kobyliak, O. Virchenko, T. Falalyeyeva, *Nutr. J.* **2015**, *15*, 43.
[24] R. E. Moreira Júnior, L. M. de Carvalho, D. C. dos Reis, G. D. Cassali, A. M. C. Faria, T. U. Maioli, A. L. Brunialti-Godard, *J. Nutr. Biochem.* **2021**, *92*, 108622.
[25] C. Kong, R. Gao, X. Yan, L. Huang, H. Qin, *Nutrition* **2019**, *60*, 175.

[26] Y. K. Lee, *Biosci. Microbiota, Food Heal.* **2013**, *32*, 12.
[27] A. Santacruz, A. Marcos, J. Wärnberg, A. Martí, M. Martin-Matillas, C. Campoy, L. A. Moreno, O. Veiga, C. Redondo-Figuero, J. M. Garagorri, C. Azcona, M. Delgado, M. García-Fuentes, M. C. Collado, Y. Sanz, EVASYON Study Group, *Obesity* **2009**, *17*, 1906.
[28] Q. Tang, G. Jin, G. Wang, T. Liu, X. Liu, B. Wang, H. Cao, *Front. Cell Infect. Microbiol.* **2020**, *10*, 151.
[29] S. Liu, P. Qin, J. Wang, *Microorganisms* **2019**, *7*, 176.
[30] S. Herp, S. Brugiroux, D. Garzetti, D. Ring, L. M. Jochum, M. Beutler, C. Eberl, S. Hussain, S. Walter, R. G. Gerlach, H. J. Ruscheweyh, D. Huson, M. E. Sellin, E. Slack, B. Hanson, A. Loy, J. F. Baines, P. Rausch, M. Basic, A. Bleich, D. Berry, B. Stecher, *Cell Host Microbe* **2019**, *25*, 681.
[31] N. R. Shin, T. W. Whon, J. W. Bae, *Trends Biotechnol.* **2015**, *33*, 496.
[32] U. Etxeberria, N. Arias, N. Boqué, M. T. Macarulla, M. P. Portillo, F. I. Milagro, J. A. Martinez, *Benef. Microbes* **2015**, *6*, 97.