Influence of Saskatoon Berry and Cyanidin-3-Glucoside on Gut Microbiota and Relationship with Metabolism and Inflammation in High Fat-High Sucrose Diet-Induced Insulin Resistant Mice

Fei Huang
Hogskolen i Innlandet Fakultet for helse- og sosialvitenskap
Ruozhi Zhao
University of Manitoba Faculty of Health Sciences
Mi Xia
Sun Yat-Sen University
Garry Shen (garry.shen@umanitoba.ca)
University of Manitoba Faculty of Health Sciences https://orcid.org/0000-0001-6947-6400

Research

Keywords: Saskatoon berry, cyanidin-3-glucoside, high fat-high sucrose diet induced insulin resistant mice, gut microbiota, inflammation

Posted Date: July 6th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-36586/v1

Abstract

Background

Type 2 Diabetes (T2D) has become one of most common and harmful chronic diseases worldwide. T2D is characterized as insulin resistant and is often associated with unhealthy dietary habits. The present study assessed the effects of freeze-dried Saskatoon berry powder (SBp) and cyanidin-3-glucoside (C3G, an anthocyanin enriched in SBp) on metabolism, inflammatory markers and gut microbiota in high fat-high sucrose diet (HFHS) diet induced insulin resistant mice.

Results

Male C57 BL/6J mice received control, HFHS, HFHS + SBp (8.0 g/kg body weight/day) or HFHS + C3G (7.2 mg/kg/day, equal amount of C3G in 8.0 g/kg/day SBp) diet for 11 weeks. HFHS diet significantly increased the levels of glucose, cholesterol, triglycerides, insulin resistance and inflammatory mediators in plasma. The results of 16S rRNA gene sequencing demonstrated that HFHS diet increased the ratio of *Bacteroidetes/Firmicutes* (B/F) phylum bacteria and an elevated abundance of *Muriculaceae* family bacteria in the feces of mice. SBp or C3G supplementation attenuated HFHS diet-induced disorders in metabolism and inflammatory markers, and increased B/F ratio and *Muriculaceae* abundance in mouse gut compared to HFHS diet alone. The abundance of *Muriculaceae* in the gut microbiota negatively correlated with body weight, glucose, lipids, insulin resistance and inflammatory mediators in mice. The results of functional predication analysis suggest that HFHS diet upregulated the genes of gut bacteria involved in inflammation-related cellular processes, and inhibited bacteria involved in metabolism. SBp and C3G partially neutralized the alterations induced by HFHS diet in gut microbiota implicated in metabolism or inflammation.

Conclusion

The findings of the present study suggest that SBp is a potential prebiotic food mitigating Western diet-induced disorders in metabolism, inflammation and gut dysbiosis, and C3G possibly contributes to the beneficial effects of SBp.

Introduction

Diabetes has become one of the most common metabolic disorders worldwide, and the trend of its upsurge is continuing. Nine out of ten diabetic patients in adults are type 2 diabetes (T2D), which is characterized as insulin resistance and often associated with obesity [1]. Genetic factors and multiple environmental factors are implicated in the etiology of T2D. However, the precise mechanism or treatment for T2D remains to unclear [2]. Western pattern diet or foods enriched with fat or sugar plays a crucial role in the epidemic of T2D in the world [3]. T2D is associated with low-grade chronic inflammation [4].

Accumulating lines of evidence suggest that gut microbiota plays an important role in the development of diabetes, obesity and inflammation [5]. Foods are the most common modulator of gut microbiota. Western diet-induced gut dysbiosis is associated with obesity and T2D [6].

Saskatoon berry (*Amelanchier alnifolia Nutt.*), a type of fruit-bearing shrubs, belongs to *Rosaceae* family in *Amelanchier* genus and is also known as Juneberry and service berry [7]. It natively grows in the southern Yukon, the Prairies in Canada and the west northern regions of the United States of America, and has been more recently planted in Europe [8, 9]. Saskatoon berry fruits contain more polyphenols including anthocyanins compared to other common berries, such as strawberry or blueberry [10]. At least four anthocyanins have been detected in Saskatoon berries, among them cyanidin-3-galactoside and cyanidin-3-glucoside (C3G) are most abundant, accounting for about 61–74% and 18–21%, respectively [8, 11].

Saskatoon berry fruits possess abundant antioxidant capacity [12, 13]. Previous studies in our group demonstrated that Saskatoon berry powder (SBp) was capable of reducing vascular inflammation in leptin receptor-knockout (db/db) diabetic mice. Supplementation of C3G resulted in a 3-times greater in anti-inflammatory activity than C3Ga in cultured endothelial cells [11, 14]. Our recent study demonstrated that supplementation of 5% SBp (~ 8.0 g/kg/day) attenuated high fat-high sucrose (HFHS) diet induced hyperglycemia, hyperlipidemia, insulin resistance, inflammation and gut dysbiosis in mice [15]. However, the effect of oral administration of C3G on the composition of gut microbiota, and the comparison of C3G and SBp on gut microbiota, metabolism and inflammatory mediators in mice fed with HFHS diet have not been documented.

The present study compared the effects of supplementation of C3G and SBp on circulating glucose, lipids, insulin, inflammatory markers and gut microbiota in a HFHS diet-sensitive rodent model, C57 BL/6J mice. Differences in the impact of SBp and C3G on gut microbial profile and the function prediction of the microbial genes in mice were further investigated using 16S rRNA gene sequencing and bioinformatics approaches.

Materials And Methods

Animals

Male C57 BL/6J mice (n = 32, 6 weeks of age) were obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were housed in standard plastic cages in an air-conditioned room with an alternative 12 h day/night light cycle and received regular mouse chow and tap water for 1 week for stabilization. The protocols of animal experiment have been approved by the Animal Protocol and Ethics Committee in the University of Manitoba.

Dietary intervention

Mice were randomized into 4 groups (n = 8/group, four in a cage) and receive one of following diets for 11 weeks: 1) control group receiving D12450K control diet from Research Diets Inc. (New Brunswick, NJ) contains 4.3% of diet, 19.2% of protein, 67.3% of carbohydrates in dry mass without an addition of sucrose; 2) HFHS group fed with HFHS diet (D12492, Research Diets) containing 35% of fat, 26% of protein and 26% of carbohydrates including 9% sucrose; 3) SBp group receiving HFHS diet supplemented with 8.0 g/kg body weight of SBp daily; 4) C3G group fed with HFHS diet supplemented with 7.2 mg/kg/day of C3G (equal to the amount of C3G containing in 8.0 g/kg SBp). SBp was prepared from lyophilized Smoky Saskatoon berries obtained from the Prairie Lane Saskatoon (Portage, MB) and stored at -80 °C [11]. Purified C3G was obtained from Polyphenols Inc. (Sandnes, Norway).

Animal monitoring and sample collection

Body weights and food intake of animals was assessed every 2 weeks until 10th weeks after the start of the dietary experiment. About 200 µL of blood was collected from mouse saphenous veins every 2 weeks after an overnight fasting for measuring levels of plasma glucose to monitor the development of diabetes. Mice were euthanized at 11 weeks after the onset of the dietary experiment via the inhalation of 5% isoflurane to reduce the pain of animals. Blood was withdrawn subsequently through heart puncture.

Analyses of metabolic variables

The levels of plasma glucose and cholesterol of mice were analyzed using Sekisui Diagnostics SL reagent kits (Charlottetown, PE). Plasma levels of triglycerides were measured using BioAssay Systems reagents (Hayward, CA). Insulin levels in plasma were assessed using enzyme-linked immunosorbent assay (ELISA) kits from EMD Millipore (Billerica, MA) for insulin. The homeostatic model assessment of insulin resistance (HOMA-IR) of the mice was calculated from the simultaneous samples of plasma insulin and glucose as previously described [16].

Measurement of circulating inflammatory

Monocyte chemotactic protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) were measured using ELISA kits from Thermo Fisher Scientific (Ottawa, ON) for MCP-1and Oxford Biomedical Research (Oxford, MI) for PAI-1, respectively.

Fecal sample collection

Mice were housed in a singly housed cage with fresh bedding overnight at the 10th week after the start of the dietary experiment for overnight. Fecal pellets were collected from individual cage and stored in separated tubes at -80 °C before further analysis.

Extraction and sequencing of bacteria DNA

DNA extraction was achieved using PowerFecal DNA Isolation Kit (QIAGEN, Germantown, MD). DNA was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific). Bacteria DNA in mouse feces was amplified using polymerase chain reaction (PCR) with modified primers containing 515F (5'-GTGYCAGCMGCCGCGGTAA) and 926R (5'-CCGYCAATTYMTTTRAGTTT) targeting the V4-V5 region of

bacterial DNA. A high-throughput Hamilton Nimbus Select robot and Coastal Genomics analytical gels were run to verify the quality of PCR products. Failed amplicons with spurious bands were repeatedly amplified after modifying conditions for PCR until qualified bands were produced. The PCR amplicons were normalized by using a high-throughput Charm Biotech Just-a-Plate 96-well normalization kit, then pooled to construct a library and quantified before sequencing on an Illumina MiSeq platform in the Integrated Microbiome Resource in the Dalhousie University [17].

Bioinformatic analysis and statistics

Freshly generated raw data of gut microbiota in the form of fastq file was demultiplexed according to the barcode sequences, followed by trimming using Cutadapt (version 1.17) to remove primers. Trimmed reads were imported as artifact into an open-source bioinformatics pipeline of decentralized microbiome analysis package Quantitative Insights Into Microbial Ecology 2 (QIIME2, version: 2018. 8) [18]. After joining of paired-end reads and filtering out of low-quality reads, amplicon sequence variants (ASVs) were obtained through DADA2 workflow. Taxonomies were assigned to ASVs using a Naive-Bayes approach and SILVA database. Diversity metrics (Core-metrics-phylogenetic) within QIIME2 were used for evaluating α- and β-diversity. Differences between data from multiple groups were examined using the analysis of variance(ANOVA) and post-hoc Tukey test. Significant difference was set at $p < 0.05$. Relative abundances of ASVs assigned with taxonomy in feature table were correlated with physical and clinical parameters. The phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to identify differences in predictive metagenome function. Functions of microbial genes were predicted with the use of Galaxy web application, and Kyoto Encyclopedia of Genes and Genomes [19].

Results

Effects of HFHS diet and supplementation of SBp or C3G on body weights and food intake of mice

Significant increases in body weights between mice fed with the control diet and HFHS diet with and without a supplementation of SBp or C3G were detected at ≥ 2 weeks after the start of the intervention ($p < 0.05$ or 0.01). The trend of body weight increase in mice receiving HFHS diet was continued throughout the experiment. No significant difference was detected among mice fed with HFHS diet with or without a supplementation with SBp or C3G (Fig. 1A). Daily food intake of all mice was measured at onset and every 2 weeks during the dietary experiment until 10 weeks after the start of dietary intervention. No significant difference in food intake was detected among various groups receiving different experimental diets in the present study (Fig. 1B).

Effects of SBp and C3G on glucose and lipid metabolism in HFHS diet-induced obese mice

The HFHS diet significantly increased the levels of fasting plasma glucose (FPG) in mice compared to the control diet ($p < 0.01$). The supplementation with SBp or C3G in the HFHS diet significantly lowered FPG,

compared to that in HFHS alone ($p < 0.05$ or 0.01). However, the levels of FPG in the SBp or C3G group were still significantly higher than that in the control group. No significant difference in FPG was found between the SBp and C3G group. The HFHS diet also significantly elevated the levels of cholesterol and triglycerides in plasma compared to the control diet ($p < 0.01$). Plasma cholesterol or triglyceride levels in both the SBp and C3G group were significantly lower than the HFHS group ($p < 0.01$). Cholesterol and triglyceride levels in the plasma of mice receiving HFHS diet supplemented with SBp or C3G were significantly higher than that in the control group ($p < 0.01$). No significant difference in plasma cholesterol or triglycerides was detected between the SBp and C3G groups (Fig. 2).

Effects of SBp and C3G on fasting plasma insulin and insulin resistance in mice receiving HFHS diet

Insulin resistance was assessed using HOMA-IR generated from the levels of FPG and insulin in the plasma samples simultaneously withdrawn from mice. The levels of plasma insulin and HOMA-IR in the HFHS group were significantly higher than the control group ($p < 0.01$). Supplementation with SBp and C3G significantly reduced the levels of insulin and HOMA-IR compared to that in mice fed with the HFHS diet alone ($p < 0.01$). The levels of insulin and HOMA-IR in the SBp or C3G group were still higher than that in the control group ($p < 0.01$). No significant difference in insulin or HOMA-IR was detected between the SBp and C3G group (Fig. 3A and 3B).

Effects of HFHS diet and the supplementation of SBp or C3G on circulating inflammatory mediators in mice

The HFHS diet elevated the levels of MCP-1 and PAI-1, two inflammatory markers, in the plasma of mice ($p < 0.01$). The supplementation of SBp or C3G in the HFHS diet significantly reduced the circulating levels of MCP-1 and PAI-1 compared to the HFHS diet in mice ($p < 0.01$). The levels of the inflammatory markers in mice receiving SBp or C3G diet were still significantly higher than the control group ($p < 0.01$). No significant difference in plasma MCP-1 or PAI-1 was detected between mice receiving HFHS diet supplemented with SBp or C3G (Fig. 4A and B).

Impact of supplementation of SBp or C3G on gut microbiota in HFHS diet-fed mice

The results of β-diversity analysis demonstrated that the gut microbial compositions in the stool of mice from the four dietary groups were well separated in principal component analysis (Fig. 5). No significant difference in α-diversity variables was detected in mice receiving different diets (Shannon index, chao1). Table 1 demonstrated that *Bacteroidetes* and *Firmicutes* represented the vast majority phylum bacteria in mouse feces. Mice fed with the HFHS diet had lower abundances of *Bacteroidetes*, *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia phylum bacteria*, but a higher relative abundance of *Firmicutes* phylum bacteria compared to mice receiving the control diet ($p < 0.05$ or 0.01). Supplementation with SBp or C3G to the HFHS diet augmented the abundance of *Bacteroidetes* and reduced that of *Firmicutes* in

mice feces ($p < 0.01$). The abundance of *Actinobacteria i*n the stool of the C3G group was the only type of phylum bacteria significantly different from that in SBp group ($p < 0.05$).

Table 1
Effect of different diets on the abundances of gut microbiota on phylum level. Male C57 BL/J6 mice (6 weeks of age) were randomized into 4 groups and received following diets for 11 weeks: control (CTL) group: low-fat diet; HFHS group: HFHS diet; SBp group: SBp (8.0 g/kg/day) supplemented in the HFHS diet; C3G group: C3G (7.2 mg/kg/day) supplemented in the HFHS diet. Values in the tables were expressed in mean ± SD (% of total gut microbiota, n = 8/group). *, **: $p < 0.05$ or 0.01 versus control group; +, ++: $p < 0.05$ or 0.01 versus HFHS group; ^: $p < 0.05$ versus SBp group.

Phylum bacteria	CTL (%)	HFHS (%)	SBp (%)	C3G (%)
Actinobacteria	5.66 ± 2.75	0.26 ± 0.11**	0.24 ± 0.15**	2.85 ± 1.83$^{*,+,\wedge}$
Bacteroidetes	54.28 ± 6.91	34.25 ± 7.36**	55.80 ± 6.95^{++}	48.36 ± 4.31^{++}
Firmicutes	23.59 ± 6.41	59.53 ± 8.00**	36.01 ± 5.73$^{**,++}$	42.34 ± 4.63$^{**,++}$
Proteobacteria	2.36 ± 0.71	0.68 ± 0.20**	0.81 ± 0.21**	0.63 ± 0.26**
Tenericutes	0.21 ± 0.23	0.20 ± 0.14	0.24 ± 0.16	0.46 ± 0.34
Verrucomicrobia	13.88 ± 3.81	4.96 ± 3.23**	6.78 ± 2.27**	5.29 ± 1.81**
Others	0.02 ± 0.02	0.12 ± 0.09*	0.11 ± 0.09	0.08 ± 0.03

The relative abundance of *Bacteroidetes* phylum bacteria in mice with HFHS diets was significantly lower than that in mice fed with the other three types of diets ($p < 0.01$, Fig. 6A). The relative abundance of *Firmicutes* phylum bacteria in the HFHS group was significantly higher compared to that in the other three groups ($p < 0.01$, Fig. 6B). Besides, the HFHS group also had a significantly lower ratio of *Bacteroidetes*/ *Firmicutes* (B/F) and higher in *Firmicutes*/ *Bacteroidetes* ratio compared to that in the control, SBp or C3G group ($p < 0.05$ or 0.01). The SBp group had a significantly higher B/F ratio and lower F/B ratio compared to the HFHS group. C3G supplementation induced a significantly lower F/B ratio ($p < 0.01$), but not in B/F ratio, compared to the HFHS group (Fig. 6C and 6D).

Statistical differences in the relative abundances of all types of gut family bacteria, except that of *Bifidobacteriaceae*, were detected among mice receiving various diets ($p < 0.01$, Fig. 7A). Mice fed with the HFHS diet had evidently higher relative abundances in *Erysipelotrichaceae* or *Lachnospiraceae* and lower abundance in *Muribaculaceae* family bacteria compared to mice in the other groups (heatmap in Fig. 7A). Supplementation of SBp or C3G decreased the abundance of *Lachinospiraceae*, and increased that of *Muribacculaceae* compared to HFHS diet ($p < 0.05$). However, the relative abundances of several family bacteria in the SBp group were different from that in the C3G group (see open triangles in Fig. 7A). Heatmap (Fig. 7B, right) demonstrated correlations between the relative abundance of family bacteria and some diabetes-related biochemical parameters. The abundances of *Defluviitaleaceae*, *Eggerthellaceae*, *Erysipelotrichaceae*, *Family XIII*, *Lachnospiraceae*, *Peptpcoccaceae*,

Peptostreptococcaeae, *Rununococcaceae* and *Streptococcaceae* family bacteria were positively correlated with body weights, glucose/lipid metabolism and inflammatory markers in the mice, while *Clostridiaceae 1*, *Clostridiales vadinBB60 group*, *Muribaculaceae* and *Lactobacillaceae* were negatively correlated with the physical, metabolic and inflammatory variables. The relative abundance of *Muribaculaceae* in mice in the HFHS group was the lowest among all groups. Both SBp and C3G supplementations significantly increased the abundances of *Muribaculaceae* family bacteria compared to the HFHS group.

The greatest difference in the mean proporation of family bacteria between the HFHS group and SBp or C3G group was *Muribaculaceae* family bacteria (Fig. 7C and 7D). The mean proportion of *Muribaculaceae* in the HFHS group was significantly lower than that in the SBp or C3G group ($p < 0.001$). The mean proportion of *Lachnospiraceae* was significantly higher in the HFHS group and lower in the SBp and C3G group ($p < 0.05$ or 0.001). Similar pattern of changes were detected in the mean proportion of *Erysipelotrichaceae*, which was higher in the HFHS group but lower in the SBp and C3G group. The mean proportion of *Ruminococcaceae* was significantly higher in the HFHS group compared to the SBp group ($p < 0.001$, Fig. 7C); while, no significant difference in the mean proportion of *Ruminococcaceae* was detected between the HFHS and C3G groups (Fig. 7D).

Functional predication of the changes in gut microbial genes induced by HFHS diet with and without the supplementation of SBp or C3G

Function prediction using PICRUSt analysis demonstrated that the impact of the HFHS diet and that supplemented with SBp or C3G on the abundances of genes of gut microbes in 15 classes of predicted functions in mice ($p < 0.01$, ANOVA, Fig. 8). Microbial genes related to membrane transport were the most abundant in the stool of mice in the present study. At least two types of ASV (operational taxonomic unit or OTUs) distribution among the cellular functions in mice receiving different dietary intervention were found: 1) the HFHS diet resulted in the highest microbial abundances compared to that in the control group, and the supplementation with SBp or C3G attenuated the effect of the HFHS diet; 2) mice in the control group had more abundant ASV than in the HFHS group, and the supplementation of SBp or C3G substantially increased ASV compared to the HFHS diet, but still relatively less than that in the control group. Examples for the first type of cellular functions include membrane transport and cell motility, which may be implicated in inflammation. The second type of cellular functions included the metabolism for energy, carbohydrates, amino acids, lipids, cofactors and vitamins, replication and repair, which may potentially contribute to metabolism. It is noticed that transcription was activated, and the translation pathway was suppressed in mice receiving the HFHS diet. SBp or C3G supplementation attenuated the effect of the HFHS diet in transcription or translation in mice. The findings suggest that SBp and C3G may neutralize the effect of the HFHS diet in inflammation and promote metabolism in mice via the modulation of gut microbiota.

Discussion

The major novel findings generated from the present study include: i) the supplementation of C3G significantly reduced HFHS diet-induced hyperglycemia, hypercholesterolemia, hypertriglyceridemia, insulin resistance, inflammatory markers and gut dysbiosis in mice in similar extents as those treated with SBp containing comparable amount of C3G; 2) both SBp and C3G supplementation to HFHS diet increased the abundance of *Bacteroidetes* phylum bacteria and decreased the abundance of *Firmicutes* phylum bacteria compared to HFHS diet alone; 3) supplementation with SBp or C3G increased the abundance of gut *Muribaculaceae* family bacteria in comparable intensity, and the abundance of *Muribaculaceae* bacteria in mouse feces was negatively correlated with body weights, FPG, lipids, and inflammatory markers in the mice; 4) the results of functional predication analysis suggests that the supplementation of SBp or C3G reduced the abundance of gut microbial genes involved in inflammation and enhanced gut microbial genes involved in the metabolic processes in mice receiving HFHS diet.

Previous studies demonstrated that C3G increased the translocation of glucose transporter-4 in skeletal muscle through the activation of insulin and AMP protein kinase pathway in mice [20]. C3G also augmented glucose-induced insulin secretion in INS-1E pancreatic β-cells and glucose uptake in HepG2 hepatocytes [21]. C3G inhibited high glucose-induced cholesterol accumulation in HK-2 kidney epithelial cells through the activation of LXRα pathway [22]. C3G-rich Bayberry extract reduced mitochondrial reactive oxygen species production and necrosis of INS-1 cells, and lowered blood glucose in streptozotocin-induced diabetic mice [23]. Previous studies in our laboratory demonstrated that C3G inhibited glycated low-density lipoproteins induced NADPH oxidase activation, mitochondrial dysfunction and cell viability in cultured vascular endothelial cells [24]. The inhibitory effect of C3G on endoplasmic reticulum stress markers in endothelial cells was at least 3-times stronger than the most abundant anthocyanin, cyandin-3-galacotoside, in SBp [14]. SBp supplementation significantly reduced HFHS diet-induced hyperglycemia, hypercholesterolemia, hypertriglyceridemia and insulin resistance in C57 BL/6J mice [15]. The results of the present study demonstrated that C3G induced similar hypoglycemic and hypolipidemic effects as SBp containing comparable amount of C3G in HFHS diet-induced insulin resistant mice. The findings of the present study are consistent with the previous studies on the effects of Saskatoon berry and C3G in glucose and lipid metabolism reported by our and other groups [11, 20, 23]. The findings suggest that C3G potentially plays a critical role in the metabolic benefits of SBp in glucose and lipid metabolism.

A recent study demonstrated that C3G administration normalized gut dysbiosis induced by a food contaminant, 3-chloro-1,2-propanediol, in rats, which was characterized by increased abundances of *Lachnospiraceae* and *Actinobaceria* phylum bacteria compared to 3-chloro-1,2-propanediol alone [25]. C3G is relatively stable in stomach and intestine [26], and it is converted to active metabolites, protocatechuic, vanillic, and p-coumaric acids in gastrointestinal rats with the assistance from gut microbiota [27]. The present study demonstrated that C3G inhibited HFHS diet-induced gut dysbiosis in mice. Both C3G and SBp significantly increased the abundance of *Bacteroidetes* phylum bacteria and *Muribaculaceae* family bacteria. *Muribaculaceae*, also known as *S24-7*, is a family of bacteria under *Bacteroidetes* phylum, which negatively influences cellular processes involving in the development of chronic inflammation, diabetes and obesity [28]. The findings of the present study suggest that

Muribaculaceae family bacteria may be the targets of C3G and SBp in gut microbiota for their beneficial effects on metabolism and inflammation.

Although the impact of supplementation of SBp and C3G on the abundances of gut *Bacteroidetes* phylum bacteria and *Muriculaceae* family bacteria in HFHS diet fed mice was comparable, but the effects of SBp and C3G on the compositions of at least other 8 families of gut bacteria were significantly different (Fig. 7A). For example, the abundance of *Clostriaceae 1* was significantly increased in the C3G group compared to that in the HFHS group, but its abundance was not significantly different between the SBp and HFHS group (Fig. 8). The results suggest that both SBp and C3G supplementation upregulated the abundance of *Muriculaceae* family bacteria, a type of gut bacteria negatively related diabetes and obesity, in mice gut, but their effects on the regulation of other family bacteria were not consistent, which may result from other components in SBp.

Functional prediction analysis helps to predict the potential of changes in gut microbiome based on metabolic pathways. Previous studies demonstrated that high fat diet reduced lipid metabolism including short chain fatty acids and the substrates for hepatic gluconeogenesis [29]. A recent study found that strawberry supplementation induced marked changes in functional potentials of microbial composition in db/db mice [30]. The present study has demonstrated the HFHS diet upregulated the genes of gut microbes with predicted functions related to inflammation and downregulated microbial genes related to lipids, carbohydrates, amino acid, cofactors and vitamins. SBp or C3G supplementation to HFHS diet tends to normalize the impact of HFHS diet on metabolism and inflammation in gut microbiota in mice. We noticed that HFHS diet increased the abundance of bacterial genes related to transcription, but decreased the genes of bacteria contributing to protein translation in mice, which possibly results from the inhibitory effect of HFHS diet on microbial genes related the metabolism of translation related substrates, such as amino acids and cofactors. SBp and C3G supplementation neutralized the contradictory effects of HFHS diet on microbial genes related to transcription and translation in mice (Fig. 8). The outcome of the functional prediction supports the metabolic and inflammatory changes induced by HFHS diet and the resilient capacity of SBp or C3G in HFHS diet-fed mice in the present study.

Conclusion

The present study demonstrated the influence of a functional food, Saskatoon berry, and one of its active components, C3G, on the metabolism, inflammation and gut microbiome in HFHS diet-induced insulin resistant mice. C3G supplementation resulted in similar beneficial effects as SBp on HFHS diet induced disorders in metabolism, inflammation and gut microbiota in mice. The results of functional predication analysis indicated that SBp or C3G reversed the changes in microbe genes related to comparable cellular functions induced by HFHS diet in mice. The findings suggest that SBp is a potential prebiotic food mitigating Western diet-induced disorders in metabolism, inflammation and gut dysbiosis, and C3G possibly contributes to the beneficial effects of SBp. The results of the present study may help to design clinical studies to investigate efficacy of Saskatoon berry and its active components on the prevention and management of diabetes in humans.

Abbreviations

ANOVA
analysis of variance
ASVs
amplicon sequence variants
B/F
Bacteroidetes/Firmicutes
C3G
cyanidin-3-glucoside
db/db
leptin receptor knockout (mice)
ELISA
enzyme-linked immunosorbent assay
FPG
fasting plasma glucose
HFHS
high fat-high sucrose
HOMA-IR
homeostatic model assessment of insulin resistance
MCP-1
monocyte chemotactic protein-1
PAI-1
plasminogen activator inhibitor-1
PCR
polymerase chain reaction
PICRUSt
phylogenetic Investigation of Communities by Reconstruction of Unobserved States
QIIME2
Quantitative Insights Into Microbial Ecology 2
rRNA
ribosomal ribonucleic acid
SBp
Saskatoon berry powder
T2D
type 2 diabetes

Declarations

· Ethics approval and consent.

· The animal experimental protocols have been approved by the Animal Protocol and Ethics Committee in the University of Manitoba (AC-11328). F. H., a graduate student, received required training and his involvement in the animal experiments has been approved (AC-11328). Consent for human data is not applicable to the present study.

· Consent for publication.

· Not applicable.

· Availability of data and materials.

· The datasets analyzed during the current study available from the corresponding author on reasonable request.

· Competing interests.

· The authors declare that they have no competing interests.

· Funding.

The experiments were financially supported by an operating grant from Diabetes Canada to G.X.S. (OG-3-15-4889-GS).

· Authors' contribution.

F.H. contributed to bioinformatics analysis of microbiome data, manuscript preparation and sample collection from animals; R.Z. contributed to animal experiments and biochemical analyses; M.X. provided important reagents and contributed to experiment design; G.X.S. contributed to project design, supervision and manuscript writing.

· Acknowledgements.

· The authors are grateful to the University of Manitoba for partial support of the stipend of F.H. through Graduates Enhancement of Tri-Council Stipends.

· **Author's information**. F.H. is a graduate student in the Department of Food and Human Nutritional Sciences in the University of Manitoba supervised by G.X.S.

References

1. Henning RJ. Type-2 diabetes mellitus and cardiovascular disease. Future cardiology. 2018;14:491–509.
2. Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, et al. Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes. 2017;66:241–55.
3. Bradley BHR. Dietary fat and risk for type 2 diabetes: a review of recent research. Current nutrition reports. 2018;7:214–26.
4. Kopp W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. Diabetes metabolic syndrome obesity: targets therapy. 2019;12:2221.
5. Sonnenburg JL, Bäckhed F. Diet–microbiota interactions as moderators of human metabolism. Nature. 2016;535:56–64.
6. Wen L, Duffy A. Factors influencing the gut microbiota, inflammation, and type 2 diabetes. J Nutr. 2017;147:1468S-75S.
7. Mazza G. Compositional and functional properties of saskatoon berry and blueberry. International journal of fruit science. 2005;5:101–20.
8. Mazza G. Anthocyanins and Other Phenolic Compounds of Saskatoon Berries Amelanchier alnifolia Nutt. J Food Sci. 1986;51:1260–4.
9. Gould K, Smreciu A, Wood S. Amelanchier alnifolia: saskatoon, service berry, June berry, shadbush, shadblow, indian pear, may cherry. 2013. Accessed 15, June 2020.
10. Hosseinian FS, Beta T. Saskatoon and wild blueberries have higher anthocyanin contents than other Manitoba berries. J Agric Food Chem. 2007;55:10832–8.
11. Zhao R, Le K, Li W, Ren S, Moghadasian MH, Beta T, et al. Effects of Saskatoon berry powder on monocyte adhesion to vascular wall of leptin receptor-deficient diabetic mice. J Nutr Biochem. 2014;25:851–7.
12. de Souza DR, Willems JL, Low NH. Phenolic composition and antioxidant activities of saskatoon berry fruit and pomace. Food Chem. 2019;290:168–77.
13. Lachowicz S, Oszmiański J, Seliga Ł, Pluta S. Phytochemical composition and antioxidant capacity of seven saskatoon berry (Amelanchier alnifolia Nutt.) Genotypes Grown in Poland. Molecules. 2017;22:853.
14. Zhao R, Xie X, Le K, Li W, Moghadasian MH, Beta T, et al. Endoplasmic reticulum stress in diabetic mouse or glycated LDL-treated endothelial cells: protective effect of Saskatoon berry powder and cyanidin glycans. J Nutr Biochem. 2015;26:1248–53.
15. Zhao R, Khafipour E, Sepehri S, Huang F, Beta T, Shen GX. Impact of Saskatoon berry powder on insulin resistance and relationship with intestinal microbiota in high fat–high sucrose diet-induced obese mice. J Nutr Biochem. 2019;69:130–8.
16. Miranda CL, Johnson LA, De Montgolfier O, Elias VD, Ullrich LS, Hay JJ, et al. Non-estrogenic xanthohumol derivatives mitigate insulin resistance and cognitive impairment in high-fat diet-induced obese mice. Scientific reports. 2018;8:1–17.

17. Walters W, Hyde ER, Berg-Lyons D, Ackermann G, Humphrey G, Parada A, et al. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. Msystems. 2016;1:e00009–15.
18. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature biotechnology. 2019;37:852–7.
19. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nature biotechnology. 2013;31:814.
20. Yamashita Y, Wang L, Nanba F, Ito C, Toda T, Ashida H. Procyanidin promotes translocation of glucose transporter 4 in muscle of mice through activation of insulin and AMPK signaling pathways. PLoS One. 2016;11.
21. Luna-Vital DA, de Mejia EG. Anthocyanins from purple corn activate free fatty acid-receptor 1 and glucokinase enhancing in vitro insulin secretion and hepatic glucose uptake. PloS one. 2018;13.
22. Du C, Shi Y, Ren Y, Wu H, Yao F, Wei J, et al. Anthocyanins inhibit high-glucose-induced cholesterol accumulation and inflammation by activating LXRα pathway in HK-2 cells. Drug design, development and therapy. 2015;9:5099.
23. Sun C-D, Zhang B, Zhang J-K, Xu C-J, Wu Y-L, Li X, et al. Cyanidin-3-glucoside-rich extract from Chinese bayberry fruit protects pancreatic β cells and ameliorates hyperglycemia in streptozotocin-induced diabetic mice. J Med Food. 2012;15:288–98.
24. Xie X, Zhao R, Shen GX. Influence of delphinidin-3-glucoside on oxidized low-density lipoprotein-induced oxidative stress and apoptosis in cultured endothelial cells. J Agric Food Chem. 2012;60:1850–6.
25. Chen G, Wang G, Zhu C, Jiang X, Sun J, Tian L, et al. Effects of cyanidin-3-O-glucoside on 3-chloro-1, 2-propanediol induced intestinal microbiota dysbiosis in rats. Food Chem Toxicol. 2019;133:110767.
26. Pradipta I, Kong D, Tan JBL. Natural organic antioxidants from green tea form a protective layer to inhibit corrosion of steel reinforcing bars embedded in mortar. Constr Build Mater. 2019;221:351–62.
27. Chen Y, Li Q, Zhao T, Zhang Z, Mao G, Feng W, et al. Biotransformation and metabolism of three mulberry anthocyanin monomers by rat gut microflora. Food Chem. 2017;237:887–94.
28. Volk JK, Nyström EE, van der Post S, Abad BM, Schroeder BO, Johansson Å, et al. The Nlrp6 inflammasome is not required for baseline colonic inner mucus layer formation or function. J Exp Med. 2019;216:2602–18.
29. Zhang L, Xie C, Nichols RG, Chan SH, Jiang C, Hao R, et al. Farnesoid X receptor signaling shapes the gut microbiota and controls hepatic lipid metabolism. MSystems. 2016;1:e00070-16.
30. Petersen C, Wankhade UD, Bharat D, Wong K, Mueller JE, Chintapalli SV, et al. Dietary supplementation with strawberry induces marked changes in the composition and functional potential of the gut microbiome in diabetic mice. J Nutr Biochem. 2019;66:63–9.

Figures

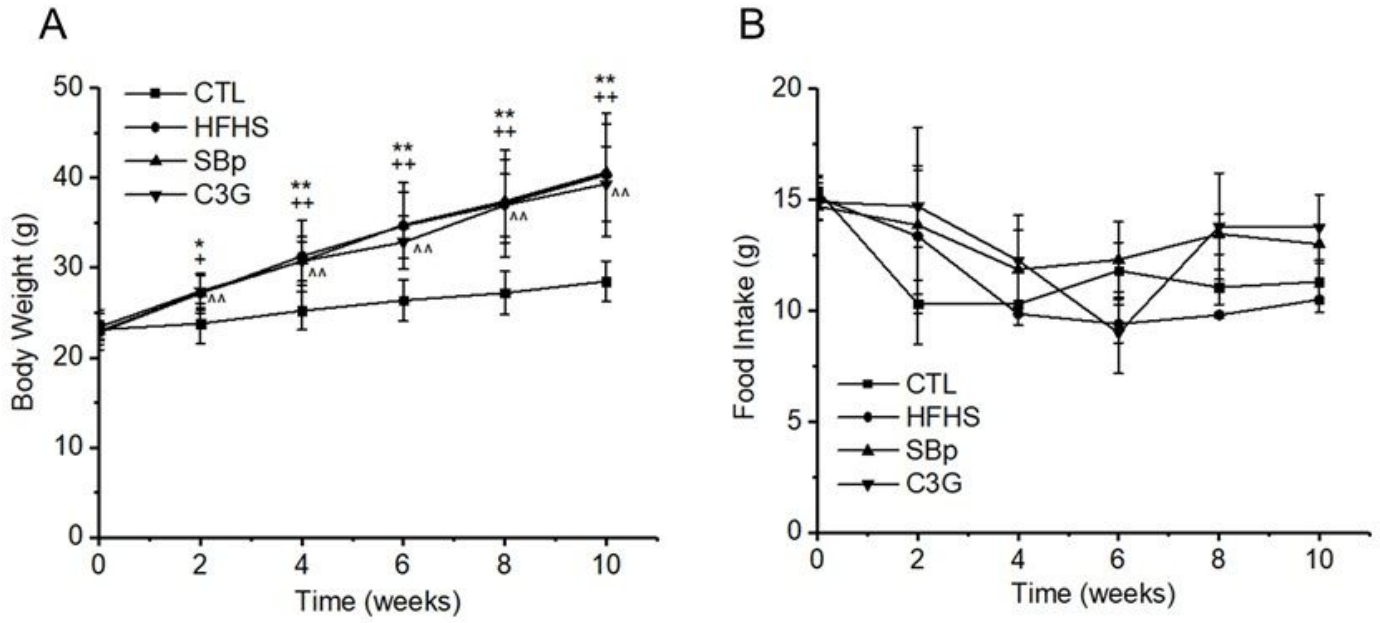

Figure 1

Effects of Saskatoon berry powder (SBp) and cyanidin-3-glucoside (C3G) supplemented in high fat-high sucrose (HFHS) diet on the body weight and food intake of mice. Male C57 BL/J6 mice (6 weeks of age) were randomized into 4 groups and received following diets for 11 weeks: control (CTL) group: control diet; HFHS group: HFHS diet; SBp group: SBp (8.0 g/kg/day) supplemented in the HFHS diet; C3G group: C3G (7.2 mg/kg/day) supplemented in the HFHS diet. Body weights and food intake were measured every two weeks up to 10 weeks. A: Body weights; B: daily food intake. The values were expressed in mean±SD g (n=8/group). *, **: $p<0.05$ or 0.01 versus CTL group.

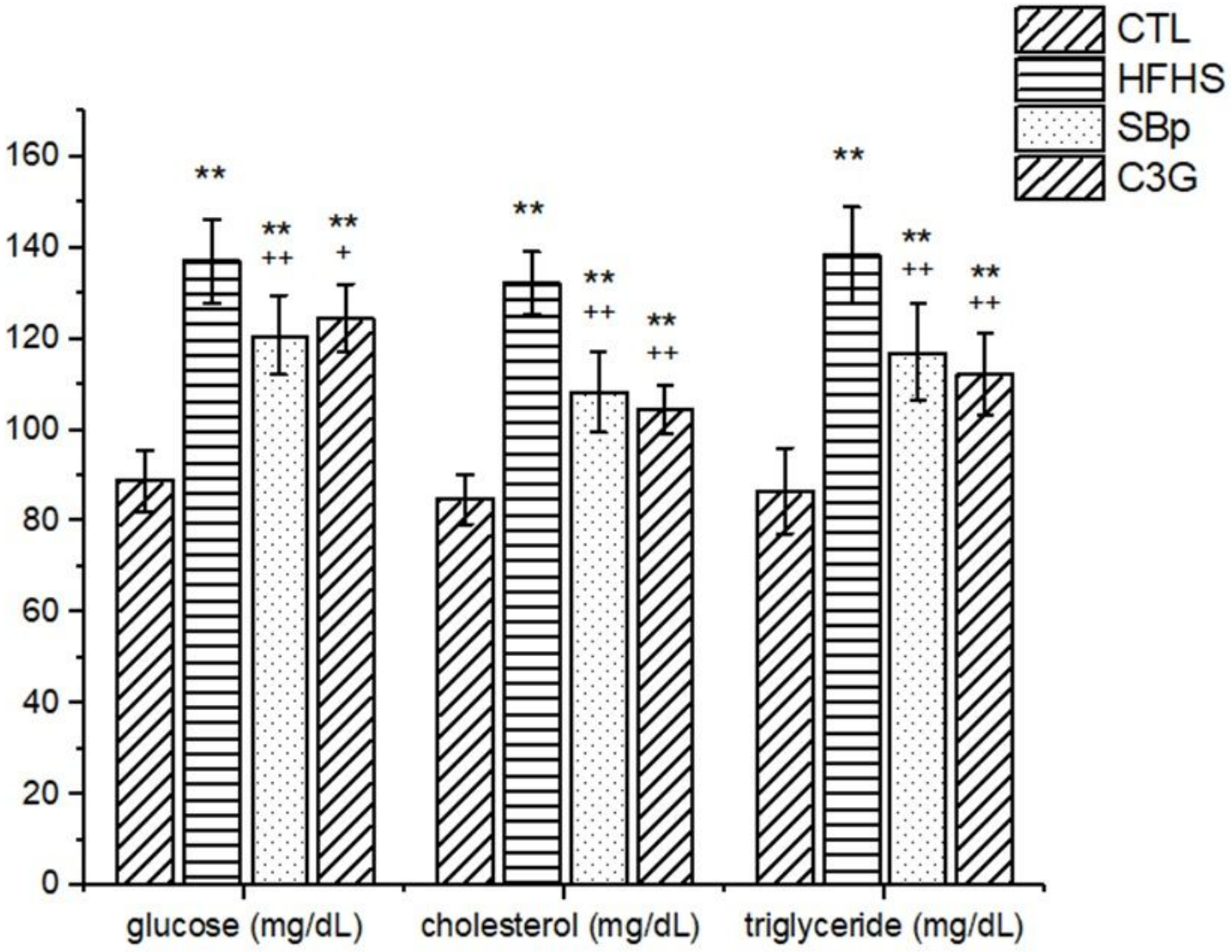

Figure 2

Levels of glucose, cholesterol and triglycerides in plasma of mice fed with HFHS diets supplemented with or without SBp or C3G. The dietary regimen was the same as described in the legend of Fig. 1. Fasting plasma glucose, cholesterol and triglycerides were measured biochemically using assay kits. Values were expressed in mean±SD mg/dL (n=8/group). *, **: $p<0.05$ or 0.01 versus the control (CTL) group; +, ++: $p<0.05$ or 0.01 versus the HFHS group.

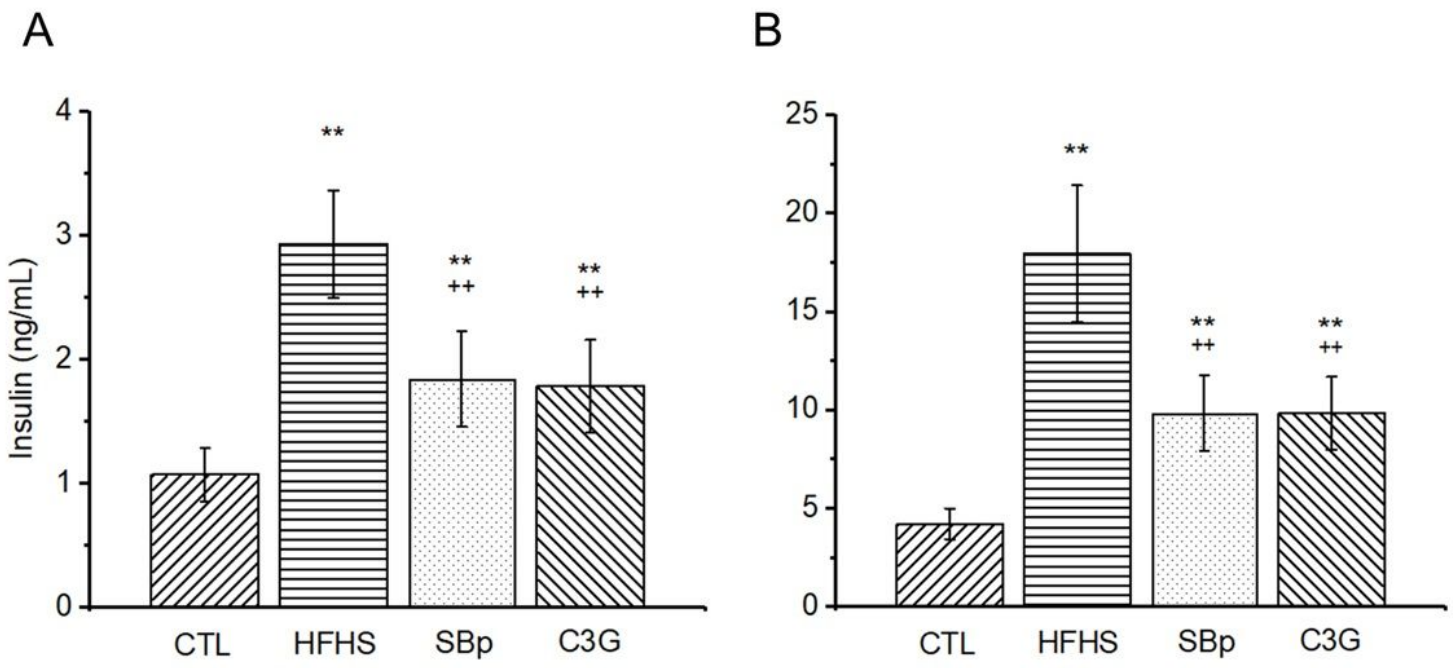

Figure 3

Effects of HFHS diets supplemented with SBp or C3G on insulin and insulin resistance in mice. The experimental regimen was described in the legend of Fig. 1. The levels of fasting plasma insulin (ng/mL) were measured at indicated time points. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated according to the levels of glucose and insulin in the same plasma samples. Values were expressed in mean±SD (n=8/group). *, **: p<0.05 or 0.01 versus the control (CTL) group; +, ++: p<0.05 or 0.01 versus the HFHS group.

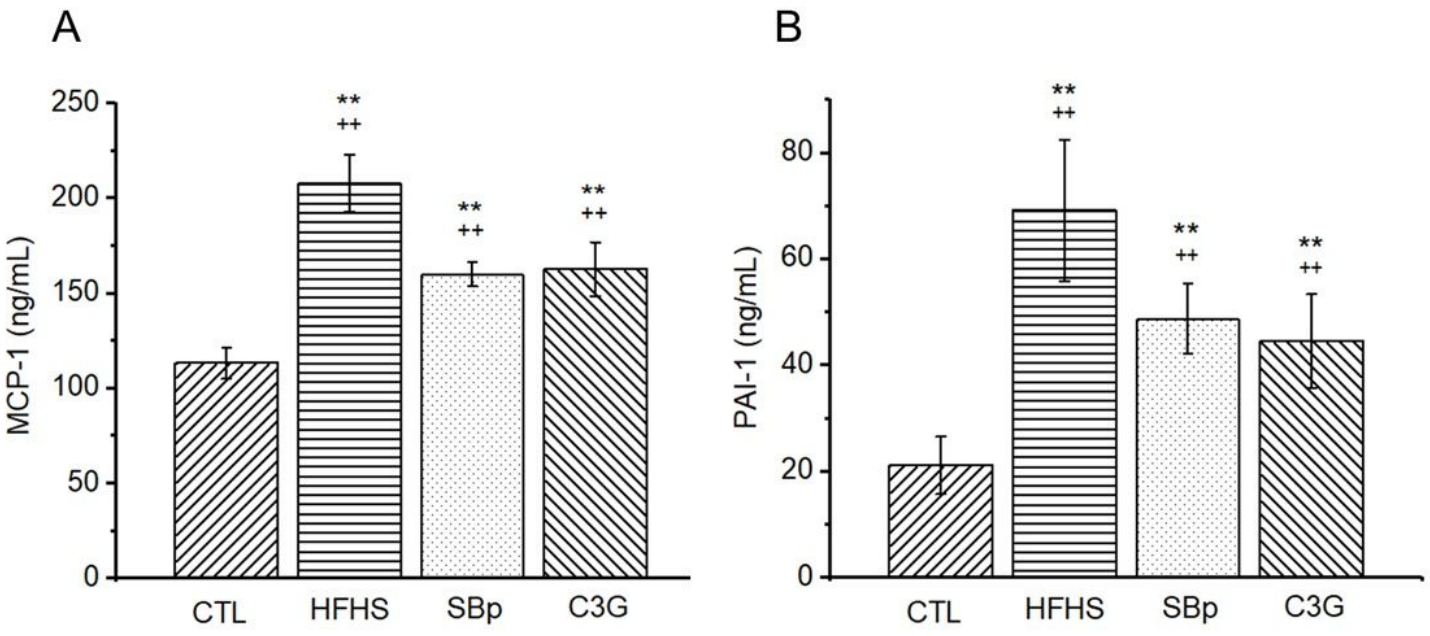

Figure 4

Levels of inflammatory regulators in plasma of mice receiving HFHS diet supplemented with SBp or C3G. The experimental regimen was described in the legend of Fig. 1. The levels of monocyte chemotactic protein-1 (MCP-1) and plasminogen activator inhibitor-1 (PAI-1) were analyzed in plasma collected before tissue harvesting using ELISA kits for mouse MCP-1 or PAI-1. Values were expressed in mean±SD ng/mL (n=8/group). *, **: p<0.05 or 0.01 versus the control (CTL) group; +, ++: p<0.05 or 0.01 versus the HFHS group.

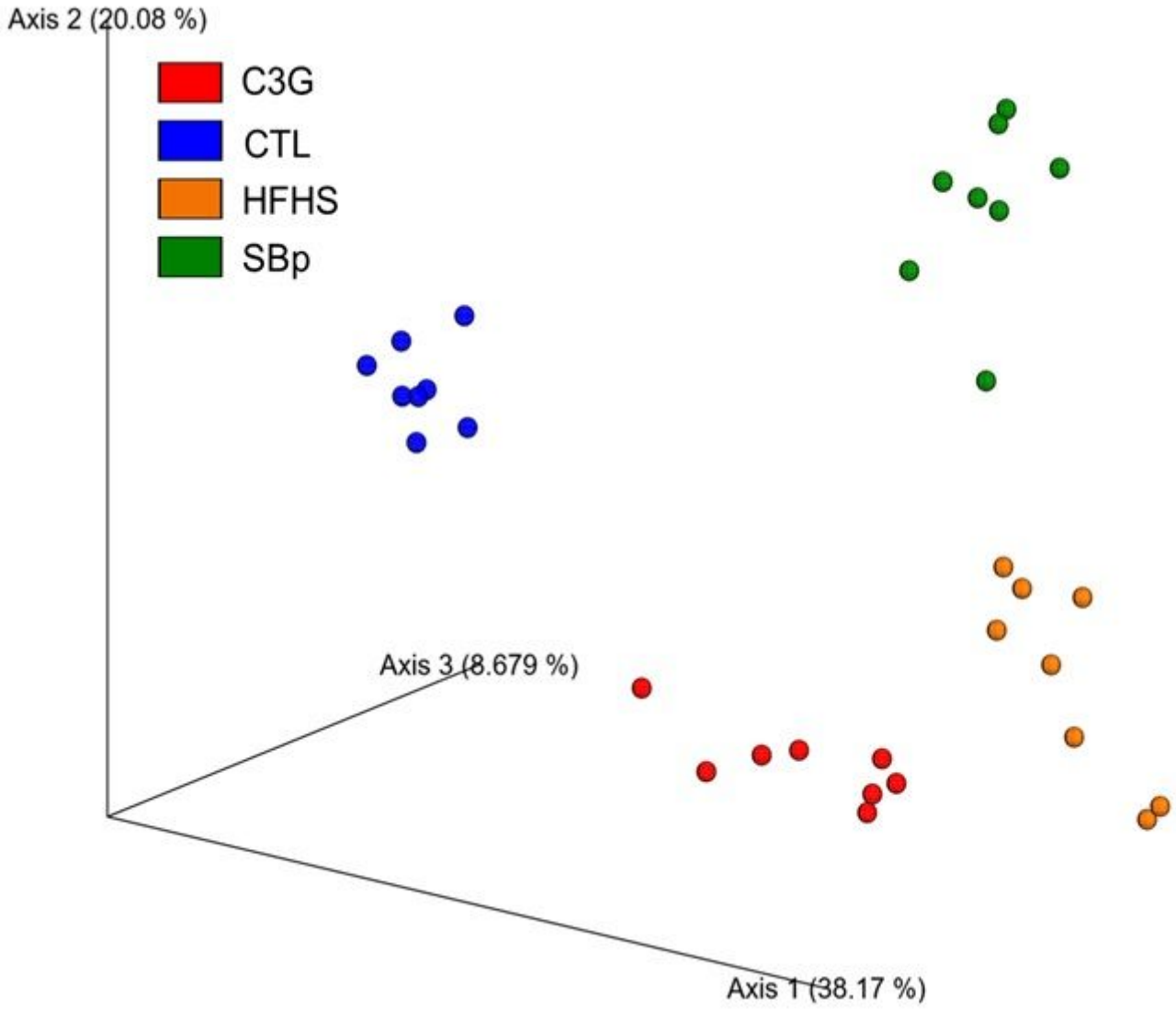

Figure 5

Effect of HFHS diet supplemented with SBp or C3G on β-diversity of gut microbiota in mice. The experimental regimen was described in in the legend of Fig. 1. Principle component analysis (PCA) was based on Bray-Curtis dissimilarities between all sample sets (weighted by taxon abundance).

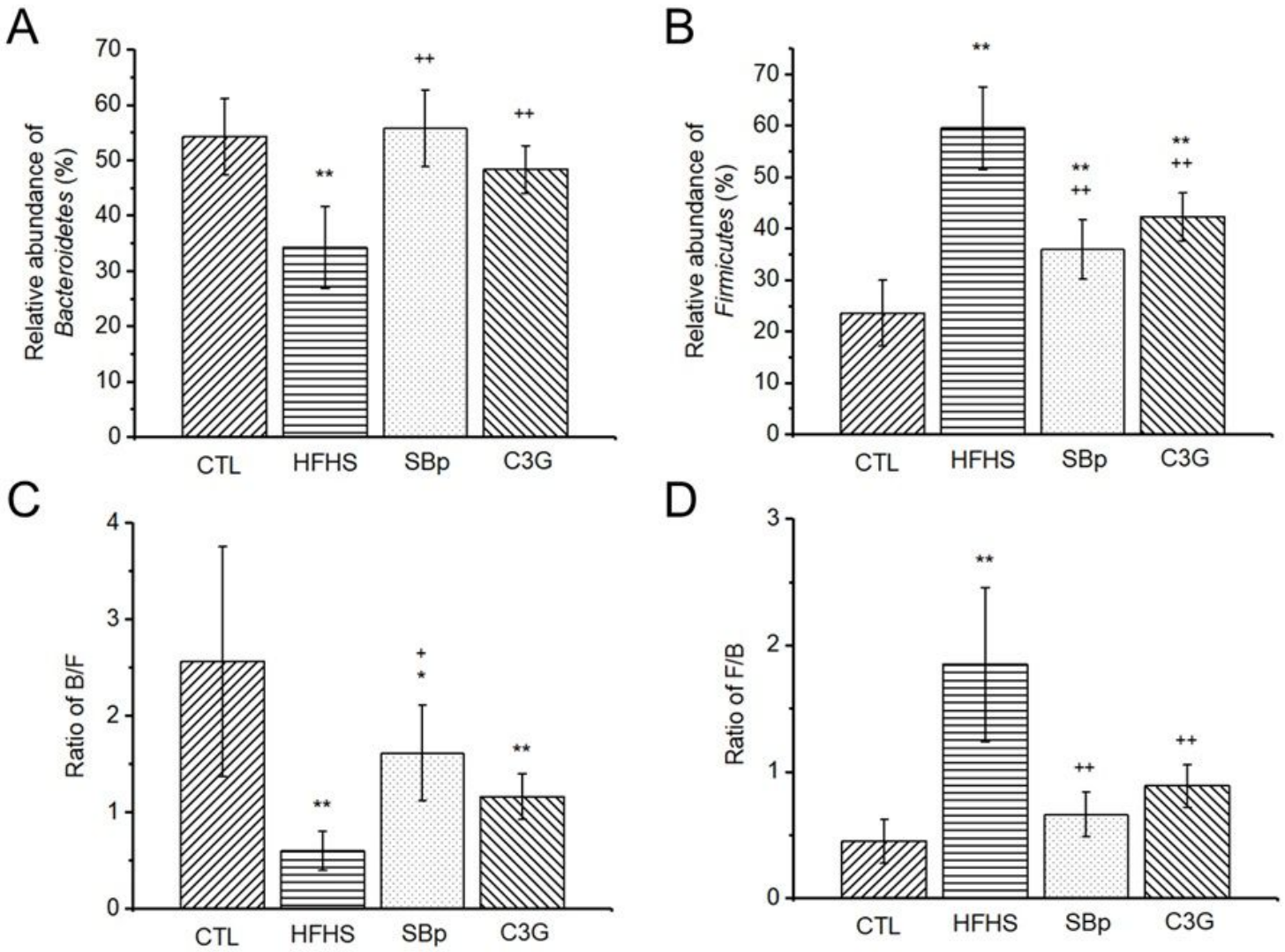

Figure 6

Effects of HFHS diet supplemented with or without SBp or C3G on the relative abundance of Bacteroidetes and Firmicutes and their ratios. The experimental regimen was described in the legend of Fig. 1. A: Relative abundance (%) of Bacteroidetes in gut microbial composition. B: Relative abundance (%) of Firmicutes in gut microbial composition. C: Ratio of Bacteroidetes over Firmicutes (B/F) in gut microbiota. D: Ratio of Firmicutes over Bacteroidetes (F/B) in gut microbiota. Values were expressed in mean±SD (%) (n=8/group). *, **: $p<0.05$ or 0.01 versus the control (CTL) group; +, ++: $p<0.05$ or 0.01 versus the HFHS group

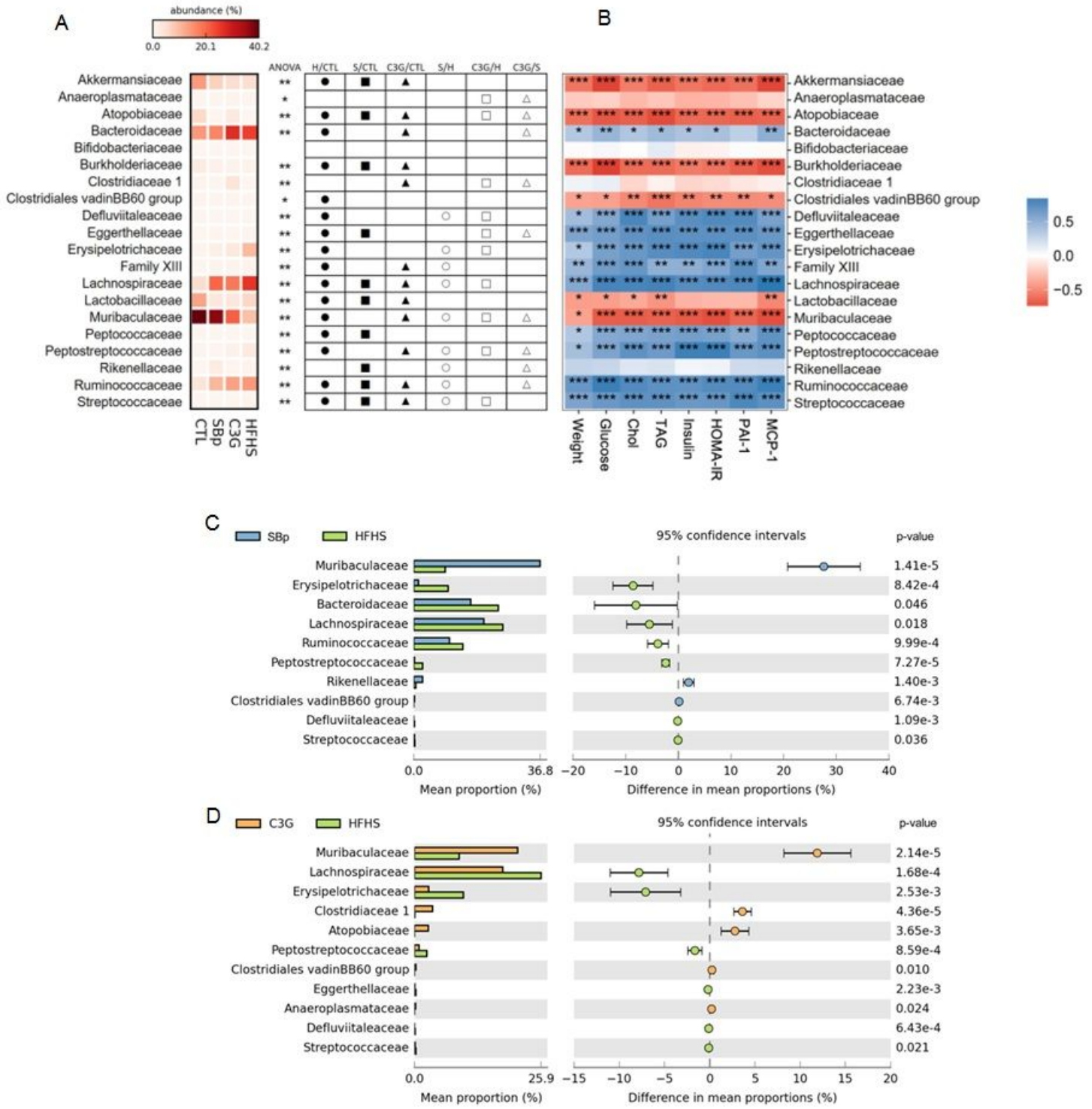

Figure 7

Effect of four HFHS (H) diets supplemented with SBp (S) or C3G on the relative abundance of gut family bacteria. The experimental regimen was described in the legend of Fig. 1. A: Statistical differences among mice with different diets (ANOVA and post-hoc Tukey test); B: correlation heatmap of relative abundance of gut microbiota on family level with physiological and biochemical parameters; C: extended error bar plot (STAMP tool) showing difference in mean relative abundance between SBp group and HFHS group. D: mean proportion and difference in mean proportion of family bacteria (STAMP tool)

between the C3G group and HFHS group (mean±SD).▯: p<0.05 in overall ANOVA result; ●: p<0.05 the HFHS (H) group versus the control (CTL) group H/CTL); ■: p<0.05 in the SBp (S) group versus the CTL group (S/CTL); ▲: p<0.05 in the C3G group versus the CTL group (C3G/CTL); ●: p<0.05 in the S group versus the H group (S/H); □: p<0.05 in the C3G group versus the H group (C3G/H); △: p<0.05 in the C3G group versus the S group (C3G/S). *, **, ***: p<0.05 or 0.01 or 0.001 in positive (blue) or negative (red) correlations between the abundance of each gut family bacteria and physiological or biochemical variables.

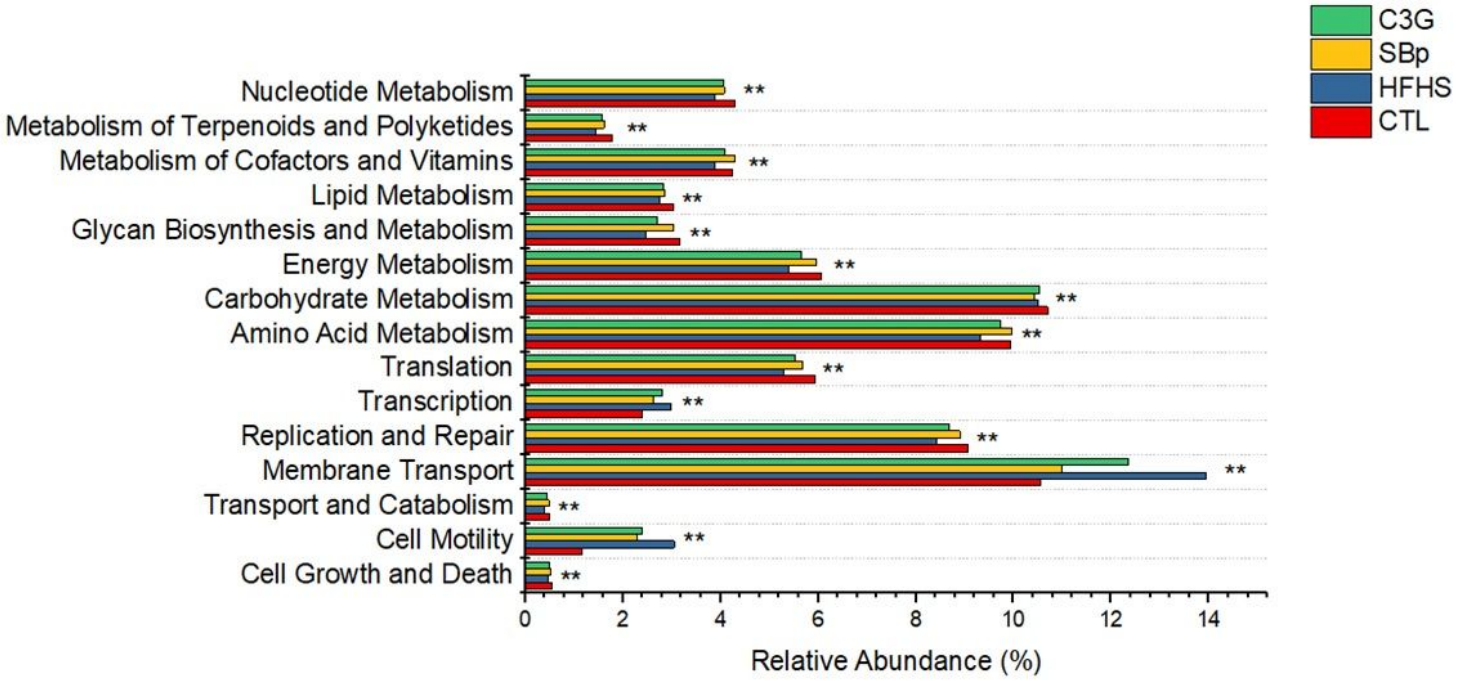

Figure 8

Effects of HFHS diets supplemented with and without SBp or C3G on functional activity in gut microbiota based on PICRUSt. The experimental regimen was the same as described in legend of Fig. 1. Differences in relative abundance (%) in each selected pathway among various dietary groups are in the form of bar plot. Values were expressed in mean value (n=8/group). *, **: p<0.05 or 0.01 showing ANOVA results among the four groups.