Effects of agavins in high fat-high sucrose diet-fed mice: an exploratory study

Alicia Huazano-García1,2, Argel Gastelum-Arellanez2,3, Juan Vázquez-Martínez4 and Mercedes G. López 5

1Hospital General Dr. Manuel Gea González, Mexico City, Mexico; 2Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico City, Mexico; 3Centro de Innovación Aplicada en Tecnologías Competitivas A.C. (CIATEC AC), León Guanajuato, Mexico; 4Superior Institute of Technology of Irapuato (ITESI), TecNM, Irapuato Guanajuato, Mexico; 5de Estudios Avanzados del IPN, Unidad IrapuatoCentro de Investigación y, Irapuato Guanajuato, Mexico

ABSTRACT

The present study was to investigate whether agavins supplementation might reduce obesity in mice fed with a high fat-high sucrose (HF-HS) diet. Mice were fed with a HF-HS diet with (HF-HS+A) and without agavins (HF-HS) supplementation. Body weight, white adipose tissue (WAT), biomarkers inflammation, gastrointestinal hormones, microbiota, and their excreted metabolites were evaluated. HF-HS+A mice significantly reduced body weight, WAT, and leptin levels compared to the HF-HS group. Furthermore, pro-inflammatory cytokines and insulin levels tended to be lower in the HF-HS+A group. Moreover, the genera Allobaculum, Akkermansia, and Sutterella, linked with positive effects on host health, were identified as possible biomarkers for agavins treatment; meanwhile, ethyl oleate, thymine, hypoxanthine, uracil, and some fatty acids were substantially enriched with agavins and negative associated with pro-inflammatory biomarkers. Collectively, these results demonstrate that agavins can ameliorate many of the harmful effects induced by intake of a diet with a high fat and sucrose content.

Efectos de las agavinas en ratones alimentados con una dieta alta en grasa y sacarosa: un estudio exploratorio

RESUMEN

El presente estudio se realizó para investigar si la suplementación con agavinas puede reducir la obesidad en ratones alimentados con una dieta alta en grasa y sacarosa (HF-HS). Los ratones fueron alimentados con una dieta HF-HS con un suplemento de agavinas (HF-HS+A) o sin dicho suplemento (HF-HS). Los parámetros evaluados fueron peso corporal, tejido adiposo blanco (WAT), biomarcadores de inflamación, hormonas gastrointestinal, microbiota y metabolitos excretados. Se constató que, en comparación con el grupo HF-HS, en los ratones HF-HS+A se redujo significativamente el peso corporal, el WAT y los niveles de leptina. Además, en este grupo las citoquinas proinflamatorias y los niveles de insulina tendieron a ser menores. Más aún, los géneros Allobaculum, Akkermansia y Sutterella, relacionados con efectos positivos en la salud del huésped, fueron identificados como posibles biomarcadores para el tratamiento con agavinas. Por otra parte, el oleato de etilo, la timina, la hipoxantina, el uracilo y algunos ácidos grasos se enriquecieron sustancialmente con las agavinas y se asociaron negativamente con biomarcadores proinflamatorios. En conjunto, estos resultados demuestran que las agavinas pueden mitigar muchos de los efectos nocivos inducidos por la ingesta de una dieta con alto contenido en grasa y sacarosa.

1. Introduction

The increment prevalence of obesity and their comorbidities have become a serious public health economic burden. Commonly, an obesogenic environment and inadequate physical activity are the main causes of the epidemic of obesity and related metabolic disorders (Ogden et al., 2007). Nevertheless, nowadays in modern human life, the high fat diet intake plus consumption of sugar-sweetened beverages is very common. In general, these foods are particularly energy-dense promoting rapid weight gain compared to balanced diets (Christ et al., 2019). In animal obesity model, it is clear that the combination of fat and sugar has a more detrimental effect, especially on insulin resistance, than saturated fat or sugar alone (Masi et al., 2017), augmenting the risk for development of type 2 diabetes as well as cardiovascular and neurodegenerative diseases (de Mello NP et al., 2019; Ormazabal et al., 2018).

A plethora of studies, in mice, have demonstrated that in relation to a standard diet, high fat-high sucrose (HF-HS) diet consumption carry out not only to an increment of body weight and adipose tissue but also to higher glucose (Gao et al., 2020; Guimarães et al., 2020; Huang et al., 2020; Li et al., 2021; Rodríguez-Daza et al., 2020), cholesterol and triglyceride levels (Guimarães et al., 2020; Huang et al., 2020; Li et al., 2021), lipid accumulation in the liver (Guimarães et al., 2020; Li et al., 2021), lipopolysaccharides (LPS) and pro-inflammatory cytokines (Gao et al., 2020; Li et al., 2021), as well as to reduction of anti-inflammatory biomarkers (Li et al., 2021) and insulin sensitivity (Gao et al., 2020; Guimarães et al., 2020; Huang et al., 2020; Li et al., 2021).
et al., 2021; Rodríguez-Daza et al., 2020). Moreover, some of these pathophysiological effects, even can appear as early as 2 weeks, after HF-HS diet intake (Yang et al., 2012). In addition, HF-HS diet consumption can affect profoundly the gut microbiota composition, commonly increasing the Firmicutes/Bacteroidetes (F/B) ratio and the relative abundance of Proteobacteria (Gao et al., 2020; Huang et al., 2020; Li et al., 2021; Rodríguez-Daza et al., 2020), as well as the overgrowth of specific taxa such as Bilophila, Helicobacter (Gao et al., 2020), Oscillospira, Ruminococcus (Rodríguez-Daza et al., 2020) and Desulfovibrio (Li et al., 2021) (to mention some). The increment of F/B ratio is associated with obesity and elevated LPS circulating levels (Gao et al., 2020; Li et al., 2021). LPS is a strong activator of toll-like receptor 4 (TLR4) which induces the expression and secretion of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1 and (IL)-6 leading to insulin resistance and dysregulated glucose homeostasis (Cani et al., 2007; Shoelson et al., 2007).

Up to date, the main focus to target obesity and related comorbidities includes restriction of caloric intake and increase of physical activity as well as the use of medications, bariatric surgery, among others (Ogden et al., 2007); however, some of these options imply high costs and may originate adverse secondary effects. In the last years, the development of dietary strategies employing prebiotics, have arisen as a good alternative for preventing or treating obesity and its comorbidities associated (Choque Delgado & Wmdsc, 2018). In this regard, inulin is perhaps the most known prebiotic; and addition of them to a HF-HS diet, substantially decrease insulin and leptin levels and increase adiponectin concentrations in rats (Sugatani et al., 2008). Recently, Igasaki et al. (2020) reported that inulin supplementation shifts the fecal microbiota composition of HF-HS diet-fed mice, decreasing the F/B ratio as well as the relative abundance of Proteobacteria.

On the other hand, agavins are a natural highly neo-branched fructans extracted from stems of Agave plants (Mancilla-Margalli & López, 2006). We previously reported that agavins supplementation can modulate the gut microbiota, with the enrichment of bacteria with huge probiotic potential such as Akkermansia in high fat diet-fed mice (Huazano-García et al., 2020). Besides, agavins not only induce changes on cecal microbiota composition but also shifts on microbial activity increasing SCFA levels (Huazano-García et al., 2017) and other metabolites (postbiotics) which could be exerting various positive metabolic effects on the host including glucose and lipid metabolism (Huazano-García et al., 2020). Since the combination of fat and sugar is an important inducer of metabolic alterations (Guimarães et al., 2020; Masi et al., 2017); in the present study, we feed mice with a high fat diet plus a high sucrose beverage (resembling to human diet) to investigate the capacity of agavins to ameliorates obesity under this dietary regimen.

2. Materials and methods

2.1. Animals

Ten male C57BL/6 mice (8 weeks old) were purchased from the Universidad Autonoma Metropolitana (Mexico City, Mexico). All mice were housed individually under temperature and humidity-controlled room with a 12-h light-dark cycles. Mice were adapted to the environment for 1 week and subsequently divided randomly in two groups and fed with a high fat diet (58Y1 Test Diet, Richmond, IN, USA) plus 30% (wt/vol) of sucrose (Sigma-Aldrich, Saint Louis, MO, USA) (HF-HS; n = 5) or supplemented with agavins (HF-HS +A; n = 5) for ten weeks. Sucrose and agavins were introduced as an extra caloric source by addition to the drinking water of mice.

The high-fat diet (58Y1 Test Diet) contained 61.6% calories from fat (31.7% lard and 3.2% soybean oil); 20.3% from carbohydrates (16.15% maltodextrin, 8.85% sucrose, and 6.46% powdered cellulose), and 18.1% from proteins. Agavins (BIOAGAVE™ powder; code: 11200001) from Agave tequilana were purchased to Ingridex Mexico. According to information provided by the supplier, each 100 g of BIOAGAVE™ contains 91.6 g of agavins (soluble fiber), 2.8 g of sugars, and 5.6% of moisture. Agavins were added in bottled water at a concentration of 0.38 g/mouse/day. The sucrose solution with or without agavins supplementation were freshly prepared daily. Clean drinking vessels were filled with an equal volume of the corresponding solution. Water intake was monitored daily to ensure all animals of the same group were drinking an equivalent volume of fluid. Because excess caloric intake is considered to be an important contributor to metabolic syndrome development or obesity; then food and water were provided ad libitum throughout the experiment. Total energy intake was obtained by the sum of the energy coming from the 58Y1 diet along with sucrose and agavins added to drinking water.

At the end of the experimental period, mice in the post-prandial state were anesthetized with a 60 mg/kg intraperitoneal dose of sodium pentobarbital to collect blood of portal vein. Subsequently, the mice were sacrificed by cervical dislocation. The cecum and adipose tissue were removed, rinsed with physiological saline and weighed. Cecum content was snap-frozen with liquid nitrogen and stored at −70°C until their use. The use of animals for this research was conducted according to the Mexican Norm (NOM-062-ZOO-1999) and approved by the Institutional Care and Use of Laboratory Animals Committee from Cinvestav-Mexico (CICUAL) protocol number: 0236–17.

2.2. Body weight gain, glucose, triglyceride, and cholesterol analysis

Body weight evolution was recorded every week along of experimental period. At the end of experimental period, blood samples from mice tails were taken in the postprandial state to measure glucose, triglycerides, and cholesterol. Blood glucose concentrations were obtained using a blood glucose meter (SD Check Gold, Mexico). Triglyceride and cholesterol analysis was carried out on serum samples using kits coupling enzymatic reaction (BioVision, Milpitas, CA, USA).

2.3. Hormones, cytokines, and lipopolysaccharides determinations

Upon sacrifice, portal blood was collected in tubes containing a dipeptidyl peptidase IV inhibitor (0.01 mL per mL of blood; Millipore, St. Louis, MO, USA) and centrifuged at 1600x g for 15 min at 4°C. Serum was stored at −80°C until
2.4. DNA extraction and next generation sequencing

Cecal genomic DNA was extracted using a QiAamp PowerFecal DNA Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Construction of a high-throughput sequencing library and Illumina-based sequencing using a MiSeq instrument was carried out by Genewiz (South Plainfield, NJ, USA). Amplicons were generated using a MetaVx™ Library Preparation kit (Genewiz, South Plainfield, NJ, USA). V3 and V4 regions of the bacterial 16S rDNA gene were amplified using forward primers containing the sequence “CCTACGGGGBGCASCAGGVRVGAAT” and reverse primers containing the sequence “GGACTACNVGGGTWTCTAATCC”. Sequencing was performed using a 2 x 150 paired-end (PE) configuration. The raw sequences generated in this study are available via the National Center for Biotechnology Information Sequence Read Archive (accession number: PRJNA772689).

2.5. Cecal microbiota analysis

Sequence data was processed and analysed on the QIME2 software (v.2020.8) (Bolyen et al., 2019). DADA2 plugin within QIME2 (Callahan et al., 2016), was used to quality filtering, dereplicating, and chimera filtering. First 18 nucleotides of forward and reverse reads were each trimmed to remove primers with ambiguous sequences. Forward reads were truncated at position 249 nt, and the reverse reads at position 241 nt based on the quality scores. We obtained a total of 1,990,271 high-quality sequences, with an average of 199,027 reads per sample, from 10 cecal samples (n = 5/group). Samples were rarefied at 91,885 sequences for alpha and beta diversity analyses. Alpha diversity was evaluated using Chao1 index (number of unique ASV) and Shannon diversity index (quantitative measure of community richness) (Gwinn et al., 2016). Differences in richness and diversity between non-supplemented control and agavins prebiotic were calculated by Kruskal-Wallis test. Beta diversity was assessed by calculating weighted UniFrac distance matrices. The distance matrices were graphically visualized by two-dimensional principal coordinates analysis (PCoA) plot. Results of the PCoA were then statistically tested by permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Generated ASV were assigned to taxonomy using the qiime feature-classifier classify-skelan feature, using a naive Bayes classifier (Pedregosa et al., 2011) trained on Greengenes v13.8 database at 99% similarity. Linear discriminant analysis (LDA) effect size (LEfSe) was used to identify taxa feature differentially expressed between non-supplemented control and agavins prebiotic (Segata et al., 2011). The threshold for the logarithmic LDA score was 3.0 with p < 0.05 for the factorial Kruskal-Wallis test.

2.6. Fecal metabolites analysis

At week ten of the experiment, feces from each mouse were collected, lyophilized, triturated, and homogenized to determine fecal metabolites profiles by Gas Chromatograph-Mass Spectrometry (GC/MS) as previously reported (Huazano-García et al., 2020). Fecal metabolites extraction was carried out using 100 mg of feces and 1 mL of chloroform/methanol (2:1). The extraction process was performed three times. Follow, the extracts were combined, and the solvent was evaporated. The residue was re-suspended in 1 mL of chloroform/methanol (2:1). An aliquot of 50 μL was taken and evaporated under nitrogen flux, after derivatized using N, O-Bis(trimethylsilyl) trifluoroacetamide with 1% trimethylchlorosilane (80 μL) and pyridine (20 μL) at 80°C for 25 min. Afterwards the system was at room temperature, isoctane was added to a final volume of 200 μL. Heptadecanoic acid was employed as an internal standard at a concentration of 3 mg/mL. One μL of the isoctane phase was injected, in a pulsed-splitless mode, onto a capillary column HP-5-MS, using He as the carrier gas at constant flow of 1 mL/min. Injector temperature was set to 260°C. The oven temperature began at 40°C (keep for 5 min) followed of an initial ramp temperature of 6°C/min until 170°C then a second ramp of 12°C/min until 290°C. The transfer line was maintained at 260°C. Mass spectrometer operated at 70 eV of electron energy, quadrupole and ion-source temperatures were 150 and 230°C respectively. All data were obtained scanning from 40–550 m/z. MassHunter Workstation software version B.0.0.6 (Agilent Technologies, Inc.) was used to collect all the data generated. Components mass spectra and retention times were obtained using the AMDIS (Automated Mass Spectral Deconvolution and Identification System) software. Fecal metabolites analysis was carried out in R 4.1.1 environment. Raw data were normalized and transformed. A principal component analysis (PCA) was applied to pre-processed data set employing the ade4 package (Dray & Dufour, 2007); differences between cluster separations on PCA were confirmed by means of Mahalanobis distance (Md) and statistics significance by Hotelling’s T2 and F-test (Goodpaster & Kennedy, 2011). A hierarchical clustering analysis (HCA) was carried out on the PCA patterns through FactoMineR package (Le et al., 2008). Peaks with the lowest p-values (p < 0.05) on PCA1 and HCA were selected and annotated by comparing their respective extracted mass spectrum with the mass spectra of data of the NIST (National Institute of Standards and Technology, USA) library and software. A heatmap was performed with all the relevant information obtained for the metabolites analysis.

2.7. Statistical analysis

All statistical analyses, except microbiota and metabolites, were performed using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA). Results are presented as mean ± SEM. Differences between groups were assessed by Student’s t-test. Differences were considered significant at p-value <0.05. Pearson correlation was applied to assess the relationship between differential microbial taxa (at genus level) and metabolites with hormones, inflammation biomarkers, and systemic effects using R environment.
3. Results

3.1. Agavins prebiotic significantly reduces body weight gain and white adipose tissue

At the end of the dietary intervention, body weight gain was notably lower in mice that received the agavins supplementation compared to the HF-HS group (p < 0.05; Figure 1a). In addition, upon sacrifice, weighing of WAT demonstrated that agavins prebiotic lead to mice a significantly lower fat accumulation (p < 0.05; Figure 1b). Moreover, energy intake (the sum of the energy from the solid food and drinking water) did not vary significantly between HF-HS and HF-HS+A group (65.63 ± 1.62 kJ and 67.14 ± 1.67 kJ, respectively), suggesting that reduction of body weight gain and WAT were not dependent of energy intake. On the other hand, glucose and triglycerides levels exhibited a trend to be lower (approximately 10% and 53%, respectively) in mice that receiving the agavins supplement with respect to non-supplemented control group (0.05 ≤ p value <0.10; Figure 1(c,d)), while cholesterol levels were not significantly different between HF-HS and HF-HS+A treatments (4.31 ± 0.24 mM and 4.30 ± 0.11 mM, respectively).

3.2. Agavins intake remarkably increased GLP-1 and decreased leptin as well as pro-inflammatory cytokines levels

Portal GLP-1 levels showed a notably increment of about of 47% in the HF-HS+A group compared to the HF-HS group (p < 0.05; Figure 2a), whereas insulin levels in mice that received the agavins prebiotic tended to be lower in comparison with non-supplemented control group (0.05 ≤ p value <0.10; Figure 2b). Moreover, HF-HS+A mice exhibited a noticeably reduction of about of 45% in portal concentration with respect to HF-HS group (p < 0.05; Figure 2c). In addition, a lower portal LPS concentration was observed in mice that received the agavins supplementation compared to the HF-HS group, but the difference did not reach statistical significance (Figure 2d). On the other hand, no significant difference was found for IL-1α levels between both treatments (Figure 2e); nevertheless, the pro-inflammatory cytokine IL-1β concentration exhibited an important reduction in mice that received the agavins treatment with respect to non-supplemented control (p < 0.05; Figure 2f). Additionally, IL-6 levels showed a trend towards a decrease.

Figure 1. Influence of agavins consumption on body weight, white adipose tissue (WAT), glucose and triglycerides levels in high fat-high sucrose diet fed mice. (A) Body weight evolution, (B) WAT, (C) Glucose, and (D) Triglycerides. Data are shown as average ± SEM (n = 5/group). Circles and triangles in bar plot represent individual rodents. Exact p-values indicates a trend to decrease (0.05 ≤ p value <0.10; unpaired t-test). Significance difference is indicated by * (p < 0.05; unpaired t-test). (HF-HS) non-supplemented control mice and (HF-HS+A) mice fed with agavins supplementation.

Figura 1. Influencia del consumo de agavinas el peso corporal, tejido adiposo blanco (WAT), niveles de glucosa y triglicéridos en ratones alimentados con una dieta alta en grasa y sacarosa. (A) Evolución del peso corporal, (B) WAT, (C) Glucosa y (D) Triglicéridos. Los datos se muestran como la media ± SEM (n = 5/ grupo). En el gráfico de barras los círculos y triángulos representan roedores individuales. Los valores de p exactos indican una tendencia a la disminución (0.05 ≤ valor p < 0.10; prueba t independiente). La diferencia significativa se indica con * (p < 0.05; prueba t independiente). (HF-HS) significa ratones control no suplementados y (HF-HS+A) significa ratones alimentados con un suplemento de agavinas.
in HF-HS+A group in comparison to HF-HS mice, however the values were not statistically significant (Figure 2g). Noteworthy, agavins intake led obese mice to a significant decrease of about 64% TNF-α concentration compared to HF-HS group (p < 0.05; Figure 2h). While the anti-inflammatory cytokine IL-10 levels in HF-HS+A group tended to be higher when compared to HF-HS group, however the values were not statistically significant (Figure 2i).

3.3. Agavins supplementation shifted the cecal microbiota composition of mice, enriching potential probiotic bacteria

Taxonomic profiling showed that the composition of the microbiota varied among mice fed with the HF-HS diet alone and supplemented with agavins (Figure 3). As expected, bacterial richness and bacterial diversity were
reduced significantly in the HF-HS+A group compared to the HF-HS group (Appendix Figure A1), because agavins promote the enrichment of specific microbial taxa (mostly probiotics). Moreover, principal coordinate analysis (PCoA) plot showed that HF-HS+A group had a distinct bacterial community structure since it is clustered separately from non-supplemented control group, indicating that agavins consumption strongly affected gut microbial composition (Appendix Figure A1c). Overall, at phylum level, the mouse cecal microbiota was greatly dominated by Firmicutes and Bacteroidetes followed by Proteobacteria. In addition, other six minor phyla: Verrucomicrobia, Cyanobacteria, TM7,
Actinobacteria, Tenericutes, and Deferrribacteres were also present (Figure 3a). HF-HS intake led to a markedly increment of Firmicutes/Bacteroidetes (F/B) ratio and a higher relative abundance of Proteobacteria. In contrast, the supplementation of agavins significantly reduced the F/B ratio and the relative abundance of Proteobacteria (Desulfovibrionaceae and Helicobacteraceae families, including the Helicobacter genus; Figure 3(b,c)). Furthermore, in relation to the HF-HS group, agavins supplementation increased the abundance of the genera Bacteroides, Allobaculum, Akkermansia, Coprococcus, Clostridium, Dehalobacterium, and Sutterella and decreased Oscillospira, Helicobacter, Odoribacter, Mucispirillum, AF12, Ruminococcus, and Roseburia (LDA > 3.0; Figure 3d). Of note, the presence of Allobaculum, Akkermansia, and Sutterella only were detected in the cecal microbiota of HF-HS+A group; thereby, these genera could be used as biomarkers for agavins prebiotic.

3.4. Agavins consumption induced changes in the fecal metabolites profiling

Fecal metabolome profile of the agavins supplemented group was significantly different from those non-supplemented mice (Figure 4). Principal component analysis (PCA) plot showed that HF-HS and HF-HS+A groups clearly separated in the PC1 (Figure 4a). A total of 109 metabolites were detected in the mice feces. Metabolites significantly contributing to the discrimination between HF-HS and HF-HS+A groups were selected based on the PCA1 and HCA analysis; thus, 35 metabolites showed the greatest differences (p < 0.05). Nonetheless, solely 24 metabolites were annotated. Besides, through the mass spectra data we were able to assign the family to which belong four compounds (Figure 4b). In relation to HF-HS diet, agavins supplementation noticeably increased the excretion of L-leucine, ethyl oleate, hypoxanthine, pentadecanoic acid, ethyl 13-methyl-tetradecanoate, uracil as well as the fatty acids ethyl esters hexadecenoic and octadecanoic; whereas levels of L-isoleucine along with the fatty acids: palmitelaidic, cis-11-eicosenoic, 9,12-octadecadienoic, cis-9-hexadecenoic, and oleic; ribose, and sterols (24-ethylcpropanol, campesterol, stigmasterol, β-sitosterol) were decreased. Of note, we detected the presence of D-glucose only in the feces of mice fed with the prebiotic supplement. Therefore, some of these metabolites could be used as potential biomarkers for agavins administration.

3.5. Correlation between differential bacteria genera and fecal metabolites with host metabolic outcomes

In general, microbial taxa notby increased with HF-HS diet were correlated positively with body weight, WAT, triglycerides, LPS, inflammatory biomarkers, insulin, and leptin levels (Figure 5). In contrast, the genera substantially enriched with agavins supplement such as Bacteroides, Allobaculum, and Akkermansia, exhibited a significant and negative correlation to body weight, WAT, and leptin concentration as well as a strong positive association with GLP-1 levels. Moreover, Coprococcus displayed a negative correlation to WAT and a positive correlation to IL-10 levels. In addition, Dehalobacterium was negatively correlated with body weight, whereas Sutterella exhibited an inverse association with WAT, LPS, and TNF-α levels. Interestingly, both Dehalobacterium and Sutterella showed a positive correlation to IL-10 and GLP-1 levels. On the other hand, several of metabolites enriched significantly in the feces of HF-HS+A group exhibited a negative correlation with TNF-α and IL-1β levels (Figure 5). In addition, ethyl oleate as well as...
hexadecenoic acid, ethyl ester; displayed an inverse association with LPS concentration. Remarkably, octadecanoic acid, ethyl ester was negatively correlated to WAT, while pentadecanoic acid showed an inverse association to IL-6 concentration. Contrary, the majority of metabolites that were decreased in the feces of HF-HS+A group, but increased significantly in the HF-HS group, such as the acids: cis-11-eicosenoic; 9,12-octadecadienoic; cis-9-hexadecenoic; and oleic showed a significant and positive correlation with insulin and leptin levels.

4. Discussion

Previous investigations have reported that dietary fat plus sucrose intake is associated with higher body weight gain and WAT as well as increase of leptin, LPS, pro-inflammatory cytokines and deteriorated insulin function (Gao et al., 2020; Huang et al., 2020; Li et al., 2021; Yang et al., 2012); which is in line with the results of the present study. Remarkably, supplementation of HF-HS diet with agavins prebiotic led to mice a substantial reduction of body weight, WAT, TNF-α, IL-1β, and leptin levels. An earlier work reported that body weight loss contributes to decreased TNF-α levels in obese individuals (Kern et al., 1995), which is in concordant with our results. In addition, WAT is known to secrete various bioactive substances that help to regulate metabolic homeostasis, such as leptin, TNF-α, IL-1β, IL-6, among others (Shoelson et al., 2007). Thus, the significant decrement of leptin, and pro-inflammatory cytokines levels observed in the HF-HS+A group could be due, in part, to the lower WAT weight. Moreover, HF-HS+A group exhibited a tendency to diminish the insulin and glucose concentration, in relation to HF-HS group, which might be associated with lower pro-inflammatory cytokines levels; because the increase of them has been associated with insulin resistance and impaired glucose homeostasis (Maedler et al., 2011; Olson et al., 2012). Interestingly, we did not find a strong effect of agavins on significant decrease of some inflammatory biomarkers such as LPS, IL-1α, and IL-6, but particularly on reduction of insulin levels as in our previous study using

![Figure 5.](image_url) Significant Pearson’s correlations between differential bacterial genera and metabolites, systemic effects, gastrointestinal hormones, and inflammatory biomarkers. The tree on the up and left illustrates a dendrogram of clustering (Ward’s method).

**Figura 5.** Correlaciones significativas de Pearson entre los géneros bacterianos diferenciales y los metabolitos, efectos sistémicos, hormonas gastrointestinales y biomarcadores inflamatorios. El árbol situado arriba y a la izquierda ilustra un dendrograma de cluster (método de Ward).
only a HF diet (Huazano-García et al., 2020); suggesting that the combination of fat and sugar exacerbated inflammation and insulin resistance (Masi et al., 2017) which is not so easy to reverse.

On the other hand, accumulating evidence indicate that perturbations in the gut microbiota composition may play an important role in the development of diseases associated with altered metabolism (Gao et al., 2020; Li et al., 2021; Rodríguez-Daza et al., 2020). In this regard, HF–HS diet notably increased the F/B ratio, which is considered as a marker of obesity (Ley et al., 2005). In addition, a remarkably increment of Proteobacteria abundance (Desulfovibrionaceae family) was also observed. The enrichment of Desulfovibrionaceae family with HF–HS diet has been previously reported (Li et al., 2021). Interestingly, some members of Desulfovibrionaceae family can produce genotoxic hydrogen sulfide (H₂S) gas leading to enhanced intestinal permeability (Rohr et al., 2020) making easy the passage of toxic metabolites into the periphery, triggering induction of pro-inflammatory cytokines.

Overall, our results strongly support that agavins supplementation had positive effects in gut microbiota modulation. Agavins intake dramatically reduced the F/B ratio and Proteobacteria proportion, which is consistent with a previous report using inulin as prebiotic in HF–HS diet-fed mice (Igarashi et al., 2020). Besides, Proteobacteria abundance show a positive correlation with LPS levels (Mi-Young et al., 2019); thereby, reduction of this bacteria phylum could be associated to decrement LPS levels in the HF–HS+A group. In addition, LPS are a strong stimulator of the release of several cytokines (Cani et al., 2007); thereby, reduction of LPS concentration in the HF–HS+A mice could be contributing to decrease IL-1β, IL-6, and TNF-α levels in these animals.

Moreover, we found that agavins supplement mostly enriched the Bacteroides genus. Interestingly, some species of this genus such as B. thetaiotaomicron, B. ovatus, and B. fragilis are emerging as novel probiotics (Chen et al., 2016; Liu et al., 2017; Tan et al., 2019). Remarkably, these Bacteroides species possess broad ability to breakdown complex polysaccharides (Flint et al., 2012). In addition to Bacteroides genus, agavins increased specific taxas such as Allobaculum, Sutterella, Akkermansia, Coprococcus, Clostridium, and Dehalobacterium. Interestingly, the substantial enrichment of all these bacteria genera agrees with an early study using inulin-type fructans as prebiotic (Everard et al., 2014). Of note, the significant increment of Bacteroides, Allobaculum, and Akkermansia is consistent with our previous work using a mice fed with a HF diet supplemented with agavins (Huazano-García et al., 2020). Moreover, in the present study, Allobaculum, Sutterella, and Akkermansia only were found in the cecal microbiota of the HF–HS+A group; thereby, these bacteria genera could be used as biomarkers for agavins treatment. Intriguingly, previous works evidenced a positive relationship between abundance of Allobaculum with a body weight reduction (Ravussin et al., 2012) and GLP-1 levels in obese mice (Huazano-García et al., 2020). Whereas Sutterella genus has been reported as important contributor to the remission of type 2 diabetes after bariatric surgery (Wang et al., 2020). Furthermore, Akkermansia is considered as a significant biomarker of gut homeostasis and host physiology, since their abundance dramatically decrease in many diseases such as obese and type 2 diabetes (Chang et al., 2019; Cheng & Xie, 2020). In addition, has been evidenced that Akkermansia reduce fat mass gain, LPS levels, and insulin resistance in obese mice (Everard et al., 2013). Recently it was reported that Akkermansia increases GLP-1 secretion, improving glucose homeostasis and ameliorating metabolic disease in high-fat diet-fed mice (Yoon et al., 2021). Thus, the presence of Allobaculum, Sutterella and Akkermansia in the cecal microbiota of HF–HS+A group, could be contributing to notably increment of GLP-1 and as well as to improve metabolic parameters. Consistently, correlation analysis revealed that Bacteroides, Allobaculum, and Akkermansia were negatively associated with body weight, WAT, and leptin concentration and positively correlated to GLP-1 levels. Moreover, Sutterella was inversely associated with WAT and LPS concentration and correlated positively with IL-10 and GLP-1 levels. Collectively, these results suggest that the agavins effects on obesity and inflammation could be mediated by gut microbiota modulation.

On the other hand, we show a significant difference in the fecal metabolomic profiles between HF–HS and HF–HS+A groups. Intriguingly, glucose was detected only in the feces of HF–HS+A group, and since this compound is mostly absorbed in the small intestine, when coming from diet (Chen et al., 2016), possibly this metabolite could be derived from gut microbes’ breakdown of agavins. Besides, we observed an increment of hypoxanthine and uracil as well as a notably reduction of isoleucine in the feces of mice that received the agavins supplement. Similar results were reported in a previous study using inulin in HF–HS diet-fed rats (Guerville et al., 2019). Moreover, L-leucine, thymine, pentadecanoic acid, and octadecanoic acid, ethyl ester; were substantially enriched in the feces of HF–HS+A group and detected as possible biomarkers for prebiotic supplementation; additionally, some of these metabolites show a strong and negative correlation with pro-inflammatory cytokines (Figure 5). Remarkably, this result is consistent with our previous work, in which uracil, L-leucine, thymine, octadecanoic acid, ethyl ester; and pentadecanoic acid were identified as biomarkers for agavins consumption and negative correlated to metabolic endotoxemia, low-grade inflammation and metabolic parameters in HF diet-fed mice (Huazano-García et al., 2020). However, since the present study was mostly exploratory, whether these metabolites mediate beneficial effects to host health require further evaluation. In addition, there is a limitation in this study because we did not include a standard-diet group (healthy mice) as reference to compare the improvement on mice health derived from agavins intake.

In summary, agavins supplementation can ameliorates several of the detrimental effects induced by consumption of a diet rich in fat and sugar such as body weight, WAT, and leptin levels. Furthermore, agavins showed a trend to decrease glucose, triglycerides, some inflammatory biomarkers (LPS, IL-1α, and IL-6), and insulin levels, but without reaching statistical significance, in relation to non-supplemented control group which could be due to exacerbated inflammation and insulin resistance, typical in HF–HS diet. On the other hand, agavins notably enriched the genera Bacteroides, Coprococcus, Clostridium, and Dehalobacterium. In addition, Allobaculum, Akkermansia, and Sutterella were identified as possible biomarkers for agavins prebiotic under this dietary regimen. Moreover,
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Appendix A

Figure A1. Agavins supplementation modified the cecal microbiota composition of mice fed with a high fat-high sucrose diet. (A) Chao1 index (number of unique ASV), (B) Shannon diversity index (quantitative measure of community richness), (C) Principal coordinate analysis (PCoA) plot of cecal communities. For each experimental group (n = 5). * indicates significant difference (p < 0.05; Kruskal-Wallis test).

Apéndice Figura 1. La suplementación con agavinas modificó la composición de la microbiota cecal de ratones alimentados con una dieta alta en grasas y sacarosa. (A) Índice Chao1 (número de ASV únicas), (B) Índice de diversidad de Shannon (medida cuantitativa de la riqueza de la comunidad), (C) análisis de coordenadas principales (PCoA) de las comunidades cecales. Para cada grupo experimental (n= 5). * indica una diferencia significativa (p < 0.05; prueba de Kruskal-Wallis).