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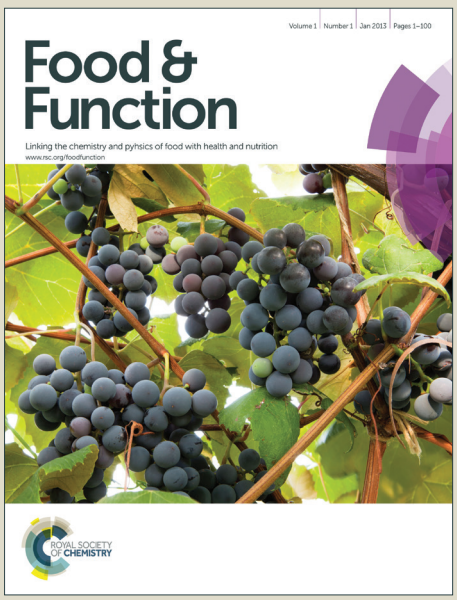

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Agavins reverse the metabolic disorders in overweight mice through the increment of short chain fatty acids and hormones

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Running title: Agavins reverse the metabolic disorders in overweight mice

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Abstract

In this study, the effects of agavins (branched fructans) along with a diet shift on metabolic parameters, short chain fatty acids (SCFA) production and gastrointestinal hormones in overweight mice were established. Male C57BL/6 mice were fed with a standard (ST) or high fat (HF) diet during 5 weeks, with the objective to induce overweight in the animals, followed by a diet shift (HF_ST) and diet shift with agavins (HF_ST+A) or inulin (HF_ST+O) for 5 additional weeks.

After the first 5 weeks, the HF group showed a 30% body weight gain and an increase in glucose, triglycerides and cholesterol concentration of 9%, 79% and 38% respectively when compared to the ST group ($P < 0.05$). Only the overweight mice that received agavins or inulin in their diets reversed the metabolic disorders induced by consumption of the HF diet, reaching values very close to those of the ST group ($P < 0.05$).

Furthermore, the consumption of agavins or inulin led to a higher SCFA concentrations in the gut and modulated hormones such as GLP-1 and leptin involved in food intake regulation ($P < 0.05$).

These findings demonstrate that a change of diet and fructans consumption such agavins is a good alternative to increase body weight lost and to improve metabolic disorders associated to overweight.

Keywords:

Agavins

Overweight

Metabolic disorders

Short chain fatty acids

Gastrointestinal hormones

Introduction

The growing prevalence of overweight and obesity is a worldwide public health problem because these conditions promote serious metabolic disorders (glucose intolerance, insulin resistance and high levels of triglycerides and cholesterol) that induce the development of type II diabetes, hypertension, dyslipidemia, cardiovascular disease and some cancers.[1,2] Changes in the dietary habits of overweight individuals or the use of prebiotics in their daily diet such fructans, may regulate lipid and glucose metabolism through the modulation of the intestinal microbiota and gastrointestinal hormones involved in appetite regulation, this might become a way to prevent and manage the risk of metabolic diseases.[3-7]

Fructans are fermented in the gut, changing the microbiota activity and its composition, promoting short chain fatty acids (SCFA) production (acetate, propionate and butyrate) and consequently reducing the luminal pH. SCFA have been established as essential nutrients that act as signaling molecules to influence glucagon-like peptide-1 (GLP-1) hormone, involved in satiety and glucose homeostasis. The ingestion of fructans has shown an increment of the L cells number in the mice proximal colon as well as the expression of proglucagon gene in those cells, leading to the secretion of different peptides, including GLP-1 that plays a relevant role on the host gut function and physiology.[8]

Cani *et al.*[9] compared the effect of the degree of polymerization (DP) of three fructans derived from chicory on GLP-1 synthesis and showed that the most important increment was observed with short DP fructans that were fermented mainly in the cecum and in the proximal gut.

Besides inulin from chicory, other important source of fructans is found in *Agave* plants endemic of Mexico. *Agave* fructans are branched carbohydrates containing

β(2-1) and β(2-6) linkages that have been classified as graminans and agavins (fructan neoseries) according to the presence of an external and internal glucose unit.[10] It is known that the complex agavins structures change with the plant age; younger plants (2 to 4 year old) have mainly short DP, while older plants (5 to 7 year old) contain principally large DP and higher complexity.[11] Regarding the role of agavins on metabolic parameters, our research group has demonstrated that agavins obtained from *Agave tequilana*, *A. angustifolia* and *A. potatorum* with a high proportion of short DP can modulate glucose and lipid metabolism as well as GLP-1 secretion on healthy mice.[12,13]

Up to now, this is the first report that assess the potential of agavins from 4 years old *Agave tequilana* plants containing a high proportion of short DP fructans. In this study, agavins were given to overweight mice to counteract metabolic disorders induced by a HF diet, to measure SCFA changes along the gut as well as the modulation of gastrointestinal hormones. We also compared the agavins effects to those of inulin.

Materials and methods

Animals and diets

Thirty-two male C57BL/6 mice (12 weeks old at the beginning of the experiment obtained from the Universidad Autonoma Metropolitana, Mexico) were individually housed in a temperature and humidity controlled room with a 12 h light-dark cycles. The mice were randomized to one of two experimental diets: a standard group (ST; n=8) was fed with a standard diet (5053, Lab Diet, USA) and a high fat group (HF; n=24) was fed with a high fat diet (58Y1; Test Diet, USA) for a 5 weeks period. At the end of this period, the HF group was divided into three new groups (n=8 per group) and a shift diet to standard diet (HF_ST) for 5 additional weeks. Two of these new groups received either agavins from *Agave tequilana* (HF_ST+A) or inulin from *Cichorium*

intybus (HF_ST+O) added in water[14-16] at a concentration of 0.38 g by mouse per day. The standard diet contained 62.4% calories from carbohydrates (starch), 24.5% from proteins and 13.1% from fat. The high fat diet had 20.3% calories from carbohydrates (16.15% maltodextrin, 8.85% sucrose, and 6.46 powdered cellulose), 18.1% from proteins and 61.6% from fat. Food and water was provided *ad libitum* throughout the experiment. All experiments were conducted according to the Guidelines of the Institutional Care and Use of Laboratory Animals Committee from Cinvestav-Mexico and according to the Mexican Norm NOM-062-ZOO-1999.

Fructans

Four-year-old *A. tequilana* Weber Blue variety plants were collected from Amatitan region, Jalisco, Mexico. *Agave* plants age corresponded to their time in the field, starting from the "*hijuelo*" (plant shoot) plantation, this material was kindly donated by Casa Cuervo S.A. de C.V. Agavins were extracted and purified in our laboratory. Firstly, the juice from the *Agave* plants was obtained using a commercial extractor. The pH juice value was adjusted to 7 using $Ca(OH)_2$. The *Agave* juice was then heated at 80-85 °C for 30 min in a water bath with continuous agitation to inactivate the hydrolytic enzymes and saponins, 1% of diatomaceous earth and activated charcoal were added to remove suspended organic impurities and coloring matter. The juice was filtered under vacuum using a nylon membrane with a pore diameter of 0.20 µm and finally lyophilized. Agavins presented an average degree of polymerization (DP) of 8[11] (Fig. S1). Linear fructans from chicory (Oligofructose; Orafti) were obtained from Megafarma® (Mexico) with an average DP of 5.

Body weight, food intake and plasma collection

Body weight was measured weekly throughout the experiment however the food intake was measured daily. The mean daily energy intake (kJ d^{-1}) was obtained by multiplying food intake (g) by the energy value of diet (kJ g^{-1}). The energy value for the ST diet was 14.28 kJ g^{-1}, for the HF diet was 21.35 kJ g^{-1} and for the agavins or inulin was 13.88 kJ g^{-1}. Blood samples after 5 and 10 weeks were taken in the postprandial state from the mice tails in order to measure glucose, triglycerides and cholesterol. Blood glucose concentrations were measured immediately using a blood glucose meter (SD Check Gold, Mexico). Blood for triglycerides and cholesterol analysis was collected in heparin tubes (0.2 ml ml^{-1} of blood) and centrifuged at 1 600g for 15 min. Plasma was stored at –80 °C until analysis, which were carried out using kits coupling enzymatic reaction (BioVision, USA). After the trial period (10 weeks) mice in postprandial state were anaesthetized by intra-peritoneal injection of sodium pentobarbital solution (60 mg kg^{-1} body weight). Blood for satiety hormone analysis was collected from the portal vein in heparin tubes containing dipeptidyl peptidase IV inhibitor (0.01 ml ml^{-1} of blood; Millipore, USA) and centrifuged at 1 600g for 15 min at 4 °C. Plasma was stored at –80 °C until analysis.

Plasma analysis for satiety hormones

GLP-1 (active), ghrelin (active), insulin and leptin concentrations were quantified using a Mouse Diabetes Standard Bio-Plex kit (Bio-Plex Pro Assay, Bio-Rad, USA) and in a Luminex instrument according to the manufacturer´s specifications. The sensitivity for the Bio-Plex kit (in pg ml^{-1}) is 0.8 for GLP-1, 0.8 for ghrelin, 22 for insulin and 6.2 for leptin.

Determination of pH and SCFA

At death, proximal, medial and distal colon segments were immediately excised. The colonic contents of each section were put in iced vials and snap frozen at –80 °C. Colonic pH measurements were made using a microelectrode (PHR-146, Lazar Research Laboratories Inc., USA). SCFA analyses were carried out following Femia *et al*.[17] protocol with some modifications, a gas chromatography and flame ionization detection from Hewlett Packard (HP4890D) was used. Briefly, 0.05 g of colon content was weighed and 0.3 ml of water was added. The solution was acidified with 0.05 ml of H_2SO_4 and SCFA were extracted by shaking with 0.6 ml of diethylether and subsequent centrifuged at 10 000g for 30 s. One microliter of the organic phase was injected directly onto a capillary column Nukol™ (30 m x 0.32 mm; Supelco, USA) at 80 °C, using N_2 as the carrier gas; detection temperature was set at 230 °C. Calibration curves of acetic, propionic and butyric acids were used to carried out SCFA quantification in the samples.

Statistical analysis

Results are presented as mean ± SEM. Differences between ST and HF groups were assessed by Student's t-test. Differences between the diets were determined using a one-way ANOVA followed by Bonferroni multiple comparison test. Differences were considered significant when $P < 0.05$. Statistical analyses were performed using GraphPad Prism (GraphPad Software, USA). Principal component analysis (PCA) was conducted using a language and environment for statistical computing R version 3.0.3 (http://www.R-project.org/) and the ade4 package.

Results

Body weight and energy intake

Initially, mice consumed a ST or HF diet for 5 weeks, mice that received the HF diet steadily gained weight, leading to a 30% weight gain with respect to the ST group (Fig. 1A and S2). After 5 weeks on the HF diet, the overweight mice[18] were shifted to a ST diet (HF_ST) or ST diet and either agavins (HF_ST+A) or inulin (HF_ST+O) addition for 5 more weeks. Only the animals that were shifted to a ST diet and received agavins or inulin in their water, showed a significant decrement on body weight ($P < 0.05$) by about 9%, surprisingly reaching values very close to those of healthy mice which were fed the ST diet throughout the whole experiment (Fig. 1B and S2). However, mice that did not receive a fructans but changed their diet (HF_ST) only showed a 4% body weight decrement ($P = 0.32$). The energy intake was significantly lower ($P < 0.05$) for mice that received the HF_ST+A and HF_ST+O diets compared to mice in the HF_ST and ST groups (Fig. 2).

Blood glucose, triglycerides and cholesterol

Mice fed 5 weeks with the HF diet, showed metabolic disorders related to glucose, triglycerides and cholesterol alterations. The HF group had significantly ($P < 0.05$) higher glucose (7.43 mM ± 0.07), triglycerides (0.95 mM ± 0.08) and cholesterol (2.44 mM ± 0.14) concentrations than the ST group (6.80 mM ± 0.05), (0.53 mM ± 0.02) and (1.77 mM ± 0.17) respectively. However, the overweight mice that were shifted to the ST diet and drank water with agavins or inulin were able to counteract the metabolic disorders induced by the HF diet consumption (Table 1). The HF_ST+A and HF_ST+O groups showed significantly lower glucose, triglycerides and cholesterol concentrations ($P < 0.05$) in relation to the HF_ST group. Interestingly, after 10 weeks, no significant

differences were found in glucose, triglycerides and cholesterol concentrations between mice that consumed prebiotics (agavins or inulin) and the mice fed with ST diet during the whole experiment (10 weeks). On the other hand, HF_ST group (overweight mice that received a shift to ST diet) was not able to counteract the metabolic disorders induced by consumption of the HF diet.

pH and SCFA in the mice gut

Mice that drank water with agavins or inulin presented a significantly pH decrement in the proximal, medial and distal gut ($P < 0.05$) compared to HF_ST and ST groups (Fig. 3A). In contrast, overweight mice that were shifted to the ST diet had a significantly pH increment in the three intestine sections ($P < 0.05$). Interestingly, only mice that consumed agavins or inulin showed an increment on SCFA concentrations along the gut in respect to HF_ST and ST groups. Agavins were fermented mostly in the medial and distal colon compared to inulin that was fermented mainly in the proximal gut (Fig. 3B, C and D). Acetic acid was the most abundant in the colon of all mice independently of the group, followed by propionic and butyric acids. There was a significant increase on acetic acid in the proximal gut of mice that drank fructans ($P < 0.05$) compared to the HF_ST and ST groups; however, in the medial gut, there were no significant differences in acetic acid between groups; whereas in the distal colon, the mice fed the ST diet throughout the 10 weeks showed a significantly lower acetic acid concentration ($P < 0.05$) compared to HF_ST+A, HF_ST+O and HF_ST groups (Fig. 3B). The amount of propionic acid was significantly higher ($P < 0.05$) in the proximal gut of HF_ST+A and HF_ST+O groups compared to HF_ST and ST groups; however, only HF_ST+A group had a significant increase of propionic acid ($P < 0.05$) in the medial gut (about 37%) and in the distal intestine (approximately 51%) compared to HF_ST+O, HF_ST and ST groups (Fig. 3C). Moreover, the mice that received agavins or inulin showed a

significantly higher concentration of butyric acid in the proximal gut ($P < 0.05$) compared to HF_ST group; finally, only the HF_ST+A group presented a significantly higher butyric acid concentration in the medial and distal segments of the intestine ($P < 0.05$) in relation to HF_ST+O, HF_ST and ST groups (Fig. 3D).

Satiety hormones response

Portal plasma GLP-1 concentrations were significantly higher in mice that drank water added with agavins or inulin ($P < 0.05$) with respect to HF_ST and ST groups (Fig. 4A); however, only overweight mice that received inulin (HF_ST+O) had significantly lower ghrelin concentration ($P < 0.05$) compared to HF_ST+A, HF_ST and ST groups (Fig. 4B). On the other hand, insulin concentrations were higher in mice that consumed either agavins or inulin ($P < 0.05$) compared to HF_ST and ST groups (Fig. 4C); Finally, leptin levels were significantly lower in mice that received agavins or inulin ($P < 0.05$) in relation to HF_ST group; interestingly, HF_ST+A and HF_ST+O groups had very similar leptin concentrations than that in the ST group (Fig. 4D).

Principal component analysis (PCA)

A PCA of all variables considered or measured in this study (body weight, glucose, triglycerides, cholesterol, SCFAs, pH and hormones) is shown in Fig. 5A. The first and second principal components (PCs) were responsible for 64% of the total variance. PC1 show a clear separation of ST and HF_ST groups but an overlap was observed for HF_ST+A and HF_ST+O groups. PC1 (42%) was controlled mainly by the butyric acid concentration in the medial intestine, propionic acid in the proximal gut and portal GLP-1 levels, whereas PC2 (22%) was controlled by triglycerides, glucose and cholesterol concentrations in the mice blood (Fig. 5B).

Discussion

In this study we evaluated the effect of HF diet consumption for 5 weeks followed by a shift to a ST diet along with fructans addition for 5 more weeks. As expected, mice on the HF diet showed a 30% increased on body weight as well as a raise in glucose (9%), triglycerides (79%) and cholesterol (38%) concentrations in the blood (features often associated with the metabolic syndrome) compared to mice that were fed with a ST diet. The results obtained on mice fed with the HF diet might be associated with a change in the gut microbiota of the animals as previously reported.[14,19,20] Moreover, it is known that a gut microbiota change as a result of a HF diet consumption is key on obesity development, insulin resistance and other metabolic syndrome hallmarks.[21,22]

Fructans are fermented in the large intestine where acetate, propionate and butyrate acids are generally produced. A clear difference on the fermentation between short DP agavins and inulin was observed (Fig. 3). Agavins with DP_{avg}=8 were slowly fermented in the proximal gut, then, a significantly increment in the medial and distal gut was observed, probably due to their intrinsic structural complexity.[10,11,23] In the other hand, inulin (linear fructans) with DP_{avg}=5 were mainly fermented in the proximal gut as reported by Cani *et al.*[9]

A pH drop in the three gut sections of both mice groups that consumed fructans was observed due to an increase on total SCFA compared to HF_ST and ST groups (Fig. 3). The pH drop might change the gut microbiota composition and promote the growth of probiotic bacteria, preventing the overgrowth of pathogenic bacteria sensitive to pH as previously reported.[24,25] In contrast, the overweight mice that only received a shift to the ST diet but no fructans showed significantly higher pH values in the three gut sections. Therefore, the solely change of diet was not sufficient to reverse the gut microbiota alterations (dysbiosis) induced by consumption of the HF diet,[26] whereas the

diet shift and fructans supplementation favorably changed the intestinal microbiota and improved overweight mice health.[14]

In this work, it was observed that only HF_ST+A and HF_ST+O groups reverted the metabolic disorders induced by the HF diet (Table 1). In other words, fructans selectively modulated the gut microbiota along the large intestine through SCFA (acetate, propionate and butyrate) production.[27]

Acetate and propionate are delivered to the liver via the portal vein where the raise in the ratio of propionate to acetate may potentially decrease lipogenesis.[7] In addition, propionic acid has been reported to inhibit fatty acid synthesis *in vitro* and have a positive influence on host metabolism by regulation of intestinal gluconeogenesis.[28] Then, the significant increment observed on propionic acid only in the gut content of overweight mice that received the agavins and inulin (Fig. 3C) can be associated with the decrement on triglycerides and cholesterol, these values were similar to those observed in healthy mice (ST group) (Table 1). Moreover, HF_ST group showed higher triglycerides and cholesterol concentrations, demonstrating or proving that a simple diet change is not enough to improve the overweight mice metabolic disorders.

On the other hand, butyrate is largely utilized in the colon by the L cells, these cells are responsible for releasing GLP-1 (potent insulinotropic hormone) which inhibits food intake (leading to reduce gain weight), lowers blood glucose, decreases glucagon secretion and enhances insulin secretion by pancreas β-cell.[29-31] Butyric acid increased significantly in the gut content of mice that consumed either fructans type. However, HF_ST+A group showed a higher butyric acid concentration in the medial and distal gut versus HF_ST+O group that presented a higher concentration of this acid in the proximal gut, this behavior might be due to the prebiotic structural differences. Besides,

292 both mice groups that consumed fructans showed a significant increment on GLP-1
293 levels in the portal vein when compared to HF_ST and ST groups (Fig. 4A). The GLP-1
294 increment in HF_ST+A and HF_ST+O might be related to the low food intake, low
295 body weight gain, glucose levels (Table 1) and the significantly higher insulin
296 concentration in the portal vein (Figure 4C).

297 Ghrelin is another hormone involved in food intake regulation, in HF_ST+O
298 group this hormone was inversely correlated to GLP-1 in portal vein.[9,32,33] Short DP
299 linear fructans are fermented preferentially in the proximal colon and butyric acid is
300 known to be responsible for the GLP-1 increment, these are key events on GLP-1
301 increment on portal vein consequently, a decrement in peripheral ghrelin.[9] HF_ST+A
302 group presented higher GLP-1 concentration (66%) and lower ghrelin (8%) but
303 HF_ST+O group had a GLP-1 increment of 53% and ghrelin reduction of 43% when
304 compared to HF_ST and ST groups (Figure 4B). The slightly reduction of ghrelin
305 concentration in HF_ST+A group might be due to the complex agavins structure, that
306 induced fermentation principally in the medial and distal intestine compared to inulin.

307 A HF diet consumption has been associated with leptin resistance and since
308 leptin is primarily involved in food intake and energy homeostasis, is also linked to the
309 regulation of glucose homeostasis and numerous gastrointestinal functions.[34]
310 Interestingly, HF_ST+A and HF_ST+O groups showed a decrease on leptin
311 concentration moreover, these values were similar to those observed for the ST group
312 (Figure 4D). The reduction of leptin levels in mice that received either agavins or inulin
313 might be also related to the lowered food intake and body weight observed in the
314 animals.

315 The PCA plot confirmed that there was a remarkable difference between the
316 overweight mice that consumed agavins or inulin and the other two mice groups (Fig.

5A). Despite the large structural differences and fermentation sites favored by HF_ST+A or HF_ST+O groups in the gut, the observed systemic effects by both fructans were similar. The PCA loading indicate that fructans consumption influenced SCFA concentration and hormones (GLP-1 and insulin) secretion. Interestingly, the body weight, ghrelin and leptin were closely associated to the HF_ST group (Fig. 5B). Finally, the PCA plot suggests that SCFAs increment, GLP-1 and insulin levels, as well as ghrelin and leptin decrement could be the most important affected parameters by HF_ST+A and HF_ST+O showing an overall decrease of the metabolic disorders (glucose, triglycerides, cholesterol and body weight) as shown in the Table 1 and Fig. 1, Fig. 3 and Fig. 4.

Conclusions

Agavins from *Agave tequilana* reverted the metabolic disorders induced by consumption of a HF diet, showing in general similar systemic effects to inulin, despite the great structural differences between fructans. Agavins reduced food intake, body weight, glucose, triglycerides and cholesterol in overweight mice, these effects were associated with the higher SCFA (propionic and butyric acid) levels in the gut content and hormones such as GLP-1, leptin and insulin in the portal vein. On the other hand, overweight mice that only were shifted to the ST diet showed a body weight loss, however, the metabolic alterations observed in these animals due to the 5 weeks on the HF diet were not revert. Therefore, a diet change along with a prebiotic consumption such agavins present a huge potential to improve the metabolic disorders associated with overweight.

Conflict of interest

The authors declare no conflicts of interest.

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References

1 S. Rössner, Obesity: the disease of the twenty-first century, *Int. J. Obes. Relat. Metab. Disord.*, 2002, **26**(suppl. 4), S2–S4.

2 D. Yach, D. Stuckler and K. D. Brownell, Epidemiologic and economic consequences of the global epidemic of obesity and diabetes, *Nat. Med.*, 2006, **12**, 62–66.

3 N. M. Delzenne, A. M. Neyrinck and P. D. Cani, Gut microbiota and metabolic disorders: how prebiotic can work?, *Br. J. Nutr.*, 2013, **109**(suppl. 2), S81–S85.

4 P. D. Cani, R. Bibiloni, C. Knauf, *et al.*, Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice, *Diabetes*, 2008, **57**, 1470–1481.

5 P. D. Cani, C. Knauf, M. A. Iglesias, *et al.*, Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor, *Diabetes*, 2006, **55**, 1484–1490.

6 E. Delmée, P. D. Cani, G. Gual, *et al.*, Relation between colonic proglucagon expression and metabolic response to oligofructose in high fat diet-fed mice, *Life Sci.*, 2006, **79**, 1007–1013.

7 C. Daubioul, N. Rousseau, R. Demeure, *et al.*, Dietary fructans, but not cellulose, decreases triglyceride accumulation in the liver of obese Zucker *fa/fa* rats, *J. Nutr.*, 2002, **132**, 967–973.

8 O. B. Chaudhri, V. Salem, K. G. Murphy, *et al.*, Gastrointestinal satiety signals, *Annu. Rev. Physiol.*, 2008, **70**, 239–255.

9 P. D. Cani, C. Dewever and N. M. Delzenne, Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats, *Br. J. Nutr.*, 2004, **92**, 521–526.

10 N. A. Mancilla-Margalli and M. G. López, Water-soluble carbohydrates and fructan structure patterns from *Agave* and *Dasylirion* species, *J. Agric. Food Chem.*, 2006, **54**, 7832–7839.

11 E. Mellado-Mojica and M. G. López, Fructan metabolism in *A. tequilana* Weber blue variety along its developmental cycle in the field, *J. Agric. Food Chem.*, 2012, **60**, 11704–11713.

12 J. E. Urías-Silvas, P. D. Cani, E. Delmée, *et al.*, Physiological effects of dietary fructans extracted from *Agave tequilana* Gto. and *Dasylirion* spp., *Br. J. Nutr.*, 2008, **99**, 254–261.

13 P. A. Santiago-García and M. G. López, Agavins from *Agave angustifolia* and *Agave potatorum* affect food intake, body weight gain and satiety-related hormones (GLP-1 and ghrelin) in mice, *Food Funct.*, 2014, **5**, 3311–3319.

14 A. Everard, V. Lazarevic, N. Gaïa, *et al.*, Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity, *ISME J.*, 2014, **8**, 1–15.

15 A. Everard, C. Belzer, L. Geurts, *et al.*, Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity, *Proc. Natl. Acad. Sci. USA*, 2013, **110**, 9066–9071.

16 A. Everard, V. Lazarevic, M. Derrien, *et al.*, Reponses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice, *Diabetes*, 2011, **60**, 2775–2786.

17 A. P. Femia, C. Luceri, P. Dolara, *et al.*, Antitumorigenic activity of the prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* on azoximethane-induced colon carcinogensis in rats, *Carcinogenesis*, 2002, **23**, 1953–1960.

18 R. T. Long, W. S. Zeng, L. Y. Chen, *et al.*, *Bifidobacterium* as an oral delivery carrier of oxyntomodulin for obesity therapy: inhibitory effects on food intake and body weight in overweight mice, *IJO*, 2010, **34**, 712–719.

19 F. Bäckhed, H. Ding, T. Wang, *et al.*, The gut microbiota as an environmental factor that regulates fat storage, *Proc. Natl. Acad. Sci. USA*, 2004, **44**, 15718–15723.

20 C. Y. Lee, The effect of high-fat diet-induced pathophysiological changes in the gut on obesity: what should be the ideal treatment?, *Clin. Transl. Gastroenterol.*, 2013, DOI: 10.1038/ctg.2013.11.

21 R. E. Ley, P. J. Turnbaugh, S. Klein, *et al.*, Microbial ecology: human gut microbes associated with obesity, *Nature*, 2006, **444**, 1022–1023.

22 P. J. Turnbaugh, R. E. Ley, M. A. Mahowald, *et al.*, An obesity-associated gut microbiota with increased capacity for energy harvest, *Nature*, 2006, **444**, 1027–1031.

23 M. G. López, N. A. Mancilla-Margalli and G. Mendoza-Diaz, Molecular structures of fructans from *Agave tequilana* Weber var. azul, *J. Agric. Food Chem.*, 2003, **51**, 7835–7840.

24 C. A. Cherrington, M. Hinton, G. R. Pearson, *et al.*, Short-chain organic acids at pH 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation, *J. Appl. Bacteriol.*, 1991, **70**, 161–165.

25 S. H. Duncan, P. Louis, J. M. Thomson, *et al.*, The role of pH in determining the species composition of the human colonic microbiota, *Environ. Microbiol.*, 2009, **11**, 2112–2122.

26 D. Hannelore, A. Moghaddas, D. Berry, *et al.*, High-fat diet alters gut microbiota physiology in mice, *ISME J.*, 2014, **8**, 295–308.

27 G. R. Gibson and M. B. Roberfroid, Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics, *J. Nutr.*, 1995, **125**, 1401–1412.

28 F. De Vadder, P. Kovatcheva-Datchary, D. Goncalves, *et al.*, Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits, *Cell*, 2014, **156**, 84–96.

29 D. J. Drucker, Biological actions and therapeutic potential of the glucagon-like peptides, *Gastroenterology*, 2002, **122**, 531–544.

30 D. J. Drucker, The biology of incretin hormones, *Cell Metab.*, 2006, **3**, 153–165.

31 J. J. Meier and M. A. Nauck, Glucagon-like peptide 1 (GLP-1) in biology and pathology, *Diabetes Metab. Res. Rev.*, 2005, **21**, 91–117.

32 P. D. Cani, A. M. Neyrinck, N. Maton, *et al.*, Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like peptide-1, *Obes. Res.*, 2005, **13**, 1000–1007.

33 P. D. Cani and N. M. Delzenne, The role of the gut microbiota in energy metabolism and metabolic disease, *Curr. Pharm. Des.*, 2009, **15**, 1546–1558.

34 Y. Ravussin, O. Koren, A. Spor, *et al.*, Responses of gut microbiota to diet composition and weight loss in lean and obese mice, *Obesity*, 2012, **20**, 738–747.

440 **Table 1** Effects on blood levels of glucose, triglycerides and cholesterol of
441 overweight mice fed with a standard diet (HF_ST), agavins or inulin
442 supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5
443 weeks; ST group are healthy mice fed with standard diet. Values are mean ±
444 SEM. Treatments with different superscript letters are significantly different ($P < 0.05$). For more details of diets and procedures, see materials and methods.

	Glucose (mM)		Triglycerides (mM)		Cholesterol (mM)	
Group	Mean	SEM	Mean	SEM	Mean	SEM
ST	6.42[b]	0.08	0.55[b]	0.01	1.67[b]	0.18
HF_ST	7.36[a]	0.11	0.82[a]	0.05	2.37[a]	0.20
HF_ST+A	6.40[b]	0.27	0.61[b]	0.02	1.89[b]	0.14
HF_ST+O	6.44[b]	0.18	0.49[c]	0.03	1.82[b]	0.12

446
447

Figure legends

Fig. 1 Body weight evolution. (A) Mice fed with a standard (ST) or high fat (HF) diet for 5 weeks. (B) Diet shift of the overweight mice to standard diet (HF_ST) and agavins (HF_ST+A) or inulin (HF_ST+O) supplement for 5 more weeks. Results are presented as mean ± SEM. Means with different letters were significantly different ($P < 0.05$).

Fig. 2 Food intake of overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks. ST group is a healthy mice fed with standard diet. Results are presented as mean ± SEM. Means with different letters were significantly different ($P < 0.05$). For more details of diets and procedures, see materials and methods.

Fig. 3 pH and SCFA concentrations in the gut content of overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks. ST group is a healthy mice fed with standard diet. (A) pH drop, (B) acetic acid, (C) propionic acid and (D) butyric acid concentrations in each of the large intestine sections. Results are presented as mean ± SEM. Means with different letters were significantly different ($P < 0.05$). For more details of diets and procedures, see materials and methods.

Fig. 4 Concentration of portal GLP-1 (A), ghrelin (B), insulin (C) and leptin (D) in overweight mice fed with a standard diet (HF_ST), agavins or inulin supplemented standard diets (HF_ST+A and HF_ST+O respectively) for 5 weeks. ST group is a healthy mice fed with standard diet. Results are presented as mean ± SEM. Means with

473 different letters were significantly different ($P < 0.05$). For more details of diets and
474 procedures, see materials and methods.
475
476 **Fig. 5** PCA. (A) Scores plot of all parameters investigated in this study in overweight
477 mice fed with a standard diet (○), standard diet supplemented with agavins (▲) or inulin
478 (◇) for 5 weeks. ST group (■) is a healthy mice fed with standard diet. (B) Loadings
479 plot the two first PCs. BW, Body Weight; GLU, Glucose; TG, triglycerides; COL,
480 cholesterol; AAP, AAM and AAD, acetic acid concentration in the proximal, medial
481 and distal gut respectively; PAP, PAM and PAD, propionic acid concentration in the
482 proximal, medial and distal gut respectively; BAP, BAM and BAD, butyric acid
483 concentration in the proximal, medial al distal gut respectively; pH P, pH M and pH D,
484 pH values in proximal, medial and distal gut respectively; GLP-1, glucagon-like
485 peptide-1; GHRE, ghrelin; INS, insulin; LEP, leptin.
486
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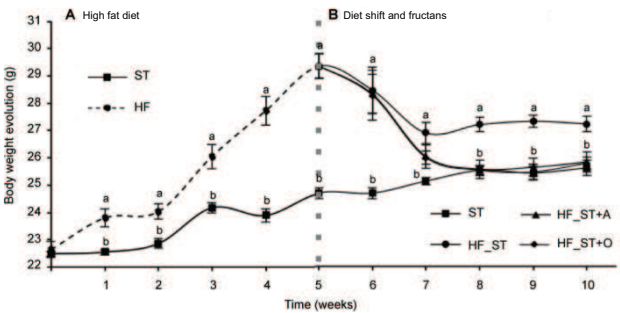
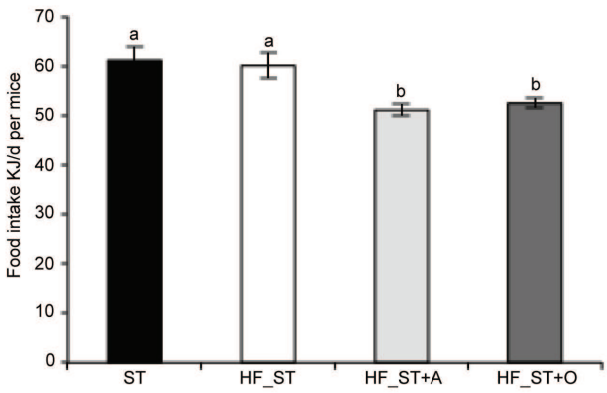
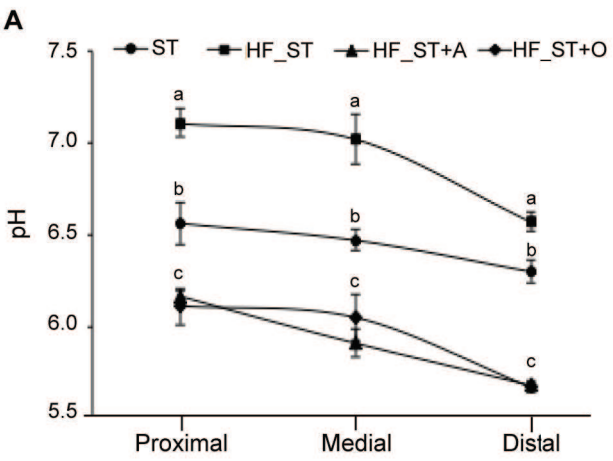
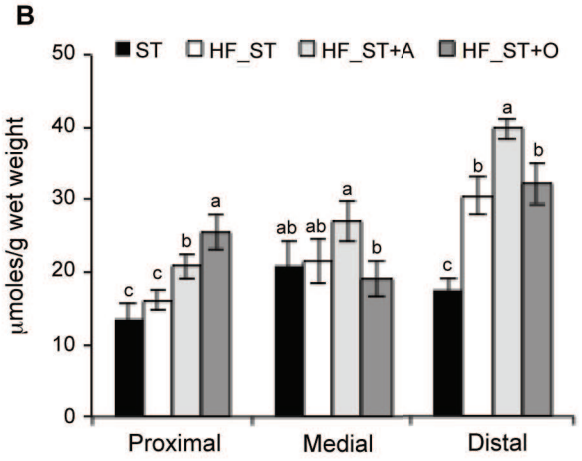
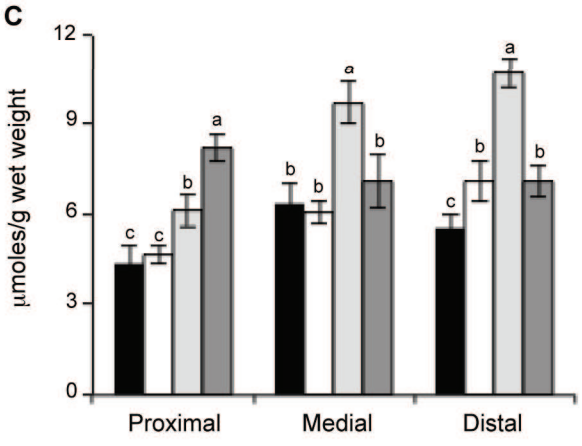
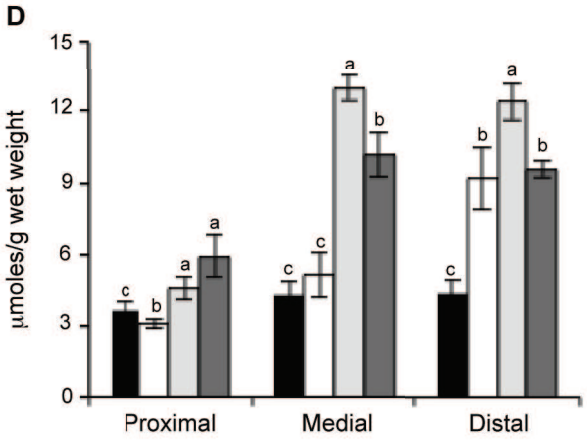


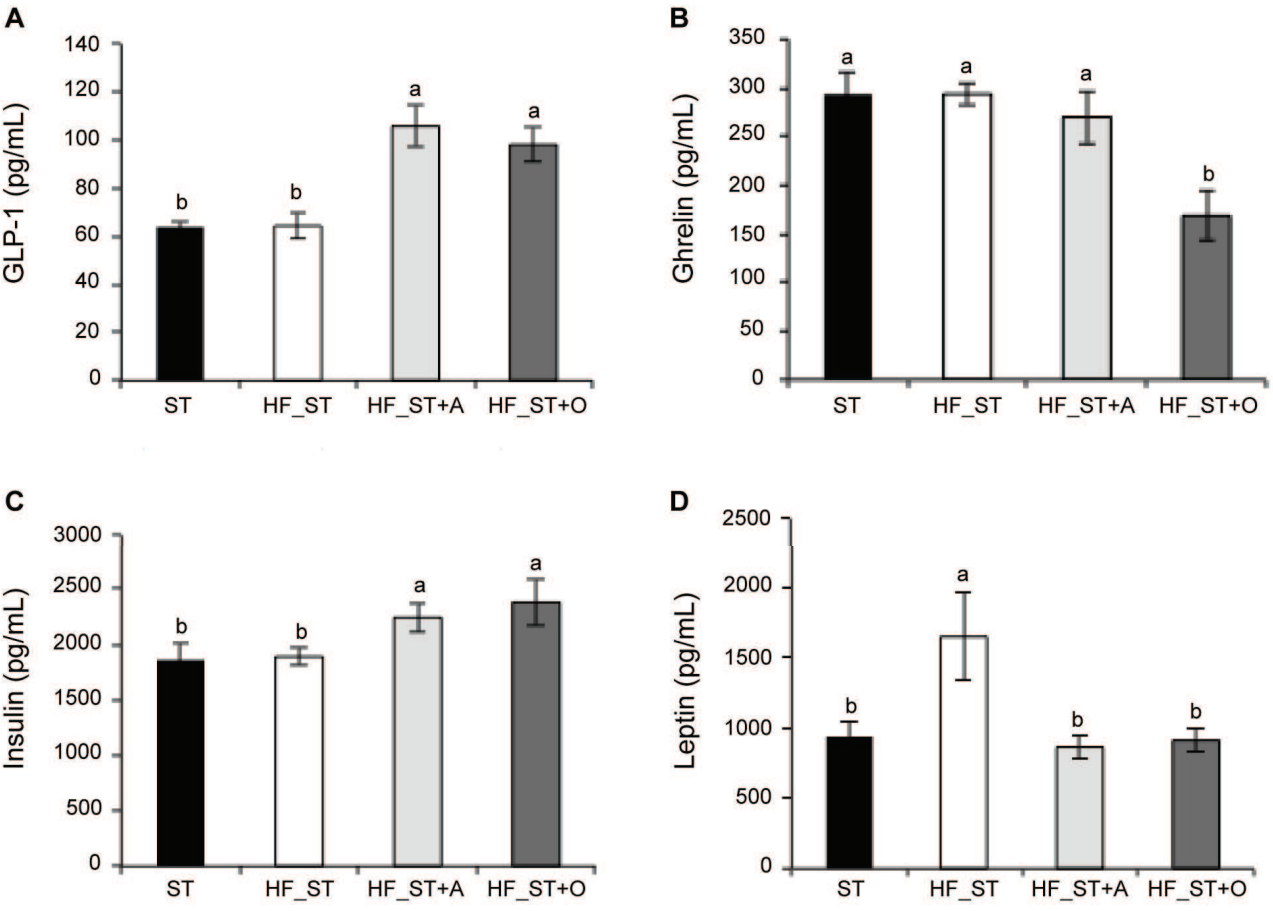


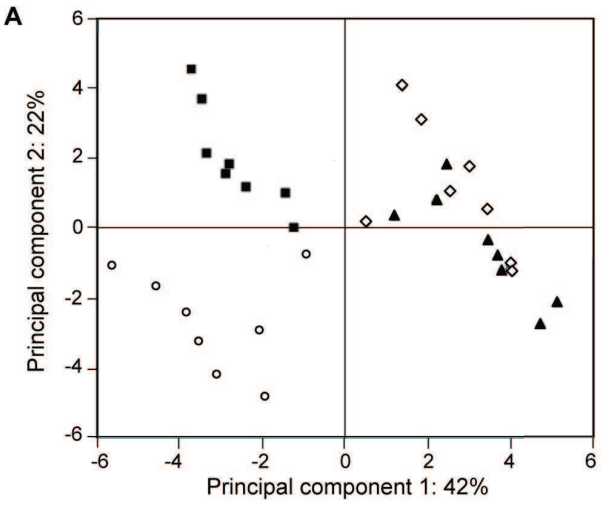


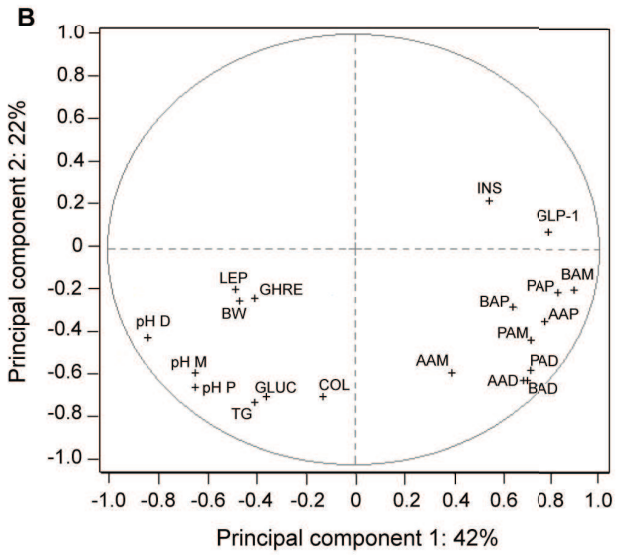


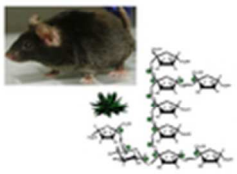










Treatment	High fat diet	Standard diet	Standard diet + agavins
	 Diet shift to:		or 
Metabolic parameters			
Body weight (g)	29.34	27.22	25.82
Glucose (mM)	7.33	7.36	6.40
Triglycerides (mM)	0.95	0.82	0.61
Cholesterol (mM)	2.37	2.37	1.89

40x20mm (300 x 300 DPI)