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ORIGINAL CONTRIBUTION

Interest of inulin in obesity: comparison of the prebiotic effect of edible-food sources *versus* purified inulin from chicory root

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Abstract

Purpose Inulin-type fructans (ITF) are fermentable dietary fibres (DF) that can confer beneficial metabolic health effects through changes in the gut microbiota. Many papers suggest that complex food rich in DF could be more relevant than purified DF in terms of health effect. We compared the prebiotic effect of natural source of inulin (scorzonera) *versus* native inulin extracted from chicory root in a model of obesity.

Methods Mice were fed during 6 weeks a low-fat (LF), high-fat (HF) or high-fat diet enriched with either purified inulin from chicory root (Inu) or lyophilized scorzonera (Sco), with the same amount of ITF intake (10%) versus a non-fermentable fibre (cellulose). Metabolic parameters were correlated with the gut microbiome composition (16S rRNA gene sequencing).

Results Both inulin sources reduced food intake without significantly modifying body weight gain or adiposity compared to HF. Purified inulin and lyophilized scorzonera differentially modulate the gut physiology and microbiota. Both inulin and scorzonera shifted global gut microbial composition from HF group, decreased members of *Desulfovibrionaceae* and boosted bifidobacteria level. Some effects were specific to Sco group, such as the increase of *Akkermansia* and the decrease of *Bacteroides,* that correlated to biological outcomes. Inu improved hepatic steatosis whereas scorzonera boosted intestinal immunity markers and antimicrobial peptides expression, and increased intestinal crypt depth.

Conclusion Differences occur between natural edible *versus* isolated sources of ITF. Both sources of inulin shifted the gut microbiota, but differently affected intestinal and lipid homeostasis. This study highlights the importance of food matrix and origins of fructans for their use in the context of metabolic disorders.

Keywords Obesity · Gut microbiota · Prebiotic · Inulin · Metabolism

Introduction

The gut microbiota appears as an important player to take into consideration in the regulation of host physiology. It is now widely recognized that alterations of the gut microbial ecosystem are associated with several pathologies including those related to nutritional and metabolic disorders (obesity, type 2 diabetes…) [1–3]. In this context, nutrition and particularly nutrients targeting the gut microbes can be of interest in the management of metabolic alterations. Some undigestible carbohydrates, defined as prebiotics, can be fermented by the gut microbiota, thereby conferring a health benefit [4]. Among them, inulin-type fructans (ITF) are certainly the most studied. ITF are well known to exhibit health benefits in humans such as a regulation of appetite [5], reduction of fat mass expansion [6] or improvement of gut barrier function [7]. ITF which have been extensively

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studied are mostly those extracted from chicory roots and consumed as lyophilized powder. We have recently focused our interest on several vegetables, fruits or cereals as potential sources of ITF [8]. Indeed, native inulin is present in a high concentration in plant roots such as Jerusalem artichoke, artichoke, asparagus, garlic, onion, leek or salsify/scorzonera [9]. However, compared to purified inulin, little is known about the metabolic effect of ITF when they are administered through vegetables naturally enriched in ITF. Few studies evaluated the impact of naturally occurring prebiotics in food on the gut microbiota composition and function, and metabolic disorders [10–12]. Our team recently demonstrated in healthy volunteers that nutritional intervention based on a selection of ITF-rich vegetables during 2 weeks (to reach a minimum intake of at least 9 g ITF/d) allows an increase in well-tolerated dietary fibre, associated with an improvement of food-related behavior such as a greater satiety and a reduced desire to eat sweet, salty and fatty food [10]. In the same cohort, we found that the intake of ITF-rich vegetables induced beneficial changes of the gut microbiota composition, such as an increased proportion of the *Bifidobacterium* genus [10]. In a multicenter and randomized placebo-controlled intervention combining both native inulin and ITF-rich vegetables, we observed that inulin-enriched diet promoted weight loss in patients with obesity and the response to the intervention was dependent of the gut microbiota composition at baseline [13, 14]. Compared to placebo, the inulin-enriched diet was more efficient to improve some clinical outcomes (weight loss, diastolic blood pressure, AST and insulinemia) and enhanced the growth of *Bifidobacterium* genus [13]. However, to our knowledge, no studies compared in the context of obesity the biological outcomes linked to identical doses of ITF given as purified compound or as vegetables. Only few vegetables display high fructans contents. In a previous study, we have shown that black salsify (*Scorzonera hispanica*) had higher content of fructans than Jerusalem artichoke (*Helianthus tuberosus*), the well-known source of ITF [15]. High fructan content was expected because both plants belong to the Asteraceae family that accumulates carbohydrates as energy storage mainly in the form of fructans [15]. We previously demonstrated that the proportion of total dietary fibre and low molecular weight soluble dietary fibre were much more important in the *Scorzonera hispanica* (salsify) than Jerusalem artichoke [15]. In this context, we hypothesized that the administration of native inulin or a preparation of ITF-rich vegetables, such as scorzonera, induces different impact on metabolic alterations versus a non-fermentable fibre (cellulose) in a model of obesity in mice.

Methods

Mice procedure

Thirty-six mice (9-week-old, C57BL/6 J, male) were purchased from Janvier Labs (Le Genest St Isle, France). Mice were housed 3 per cage in a controlled environment (12-h daylight cycle) with free access to food and water. The experiment was approved by the local ethics committee for animal care of the Health Sector of Université catholique de Louvain under the specific agreement number 2017/UCL/MD/005. Housing conditions were as specified by the Belgian Law of 29 May 2013 regarding the protection of laboratory animals (Agreement no LA 1230314). Every effort was made to minimize animal pain, suffering, and distress.

The acclimatization period lasted one week with a standard diet (Research Diet Inc., New Brunswick, NJ, USA). Mice were split in four different groups (9 mice per group). One control group was fed with a low-fat diet (LF, 10% kcal from fat, Ssniff, Soest, Germany), whereas the three other groups were fed with a high-fat diet (HF, 45% kcal from fat, Ssniff, Soest, Germany). Among the three groups fed a HF diet, one group received purified ITF (Inu, chicory powder extract from Cosucra, Warcoing, Belgium). In Inu group, 11% of inulin were added into the diet in order to obtain a final diet with 10% of fructans. Another HF group received a preparation of lyophilized scorzonera (Sco). In Sco group, HF diet was supplemented with 18% of lyophilized scorzonera in order to obtain 10% of fructans. In order to have the same percentage of all nutrients (including fat content) except the type of DF in the three HF groups after the addition of 11% of inulin (w/w) for Inu group and 18% of scorzonera (w/w) for Sco group, we adjusted the diet by incorporating cellulose, BW200 (18% in HF group and 7% in Inu group). This adjustment was necessary due to the differences in fructans content in purified inulin (92.6 g/100 g) and preparation of lyophilized scorzonera (54.5 g /100 g). As a consequence, the three groups of HF contained 82% of the initial diet and the Inu and Sco groups contained the same amount of fructans each. A detailed composition of products and diets is shown in Table 1. Scorzonera used in this study were purchased from a local grocery store and prepared according to standard cooking practises including a steaming for 20-min. Upon cooking, scorzonera were subsequently frozen at −18 °C and freeze-dried in a Christ 1022 freeze-drier (Osterode, Germany) at 0.630 mbar until constant weight was reach with an initial shelf T of −5 °C. Fructan and free sugar (glucose, fructose, sucrose) contents of the freeze-dried scorzonera samples and purified inulin extract were determined according to

Table 1 Composition and intake of diets and ITF supplements

		Purified inulin	Lyophilized scorzonera	
Supplement composition (g/100 g)				
Total dietary fibre		33.8	18.3	
Insoluble dietary fibre		0.3	9.6	
Soluble dietary fibre		33.5	8.7	
Fructans		92.56	54.54	
Free glucose		1.4	0.84	
Free saccharose		3.86	3.13	
Free fructose		1.93	2.98	
	LF	HF	Inu	Sco
Diet composition (g/100 g)				
Saccharose	0	16.54	16.54	16.54
Fat	4	19.35	19.35	19.35
Protein	20.7	20.5	20.5	20.5
Maltodextrin	14	9.02	9.02	9.02
Cellulose	5	23.7	12.7	5.7
Starch	48.85	5.74	5.74	5.74
Native inulin	0	0	11	0
Lyophilized scorzonera	0	0	0	18
Others (minerals. vitamins…)	7.45	5.15	5.15	5.15
Energy (kcal/grams of diet)	2.62	3.64	3.87	3.89
Diet consumption				
Cumulative food intake (g)	154.9 ± 4.3^{a}	139.5 ± 3.6^{a}	119.4 ± 4.6^{b}	118.7 ± 1.7^{b}
Cumulative fat intake (g)	6.2 ± 0.17^{a}	26.98 ± 0.69^{b}	23.10 ± 0.89^{c}	22.97 ± 0.32^{c}
Cumulative saccharose intake (g)	1.55 ± 0.04^{a}	23.07 ± 0.59^{b}	20.26 ± 0.78^{c}	19.64 ± 0.27^{c}
Cumulative glucose intake (g)	0 ± 0^{a}	0 ± 0^{a}	0.18 ± 0.01^{b}	0.18 ± 0.00^{b}
Cumulative fructose intake (g)	0 ± 0^{a}	0 ± 0^{a}	0.25 ± 0.01^{b}	0.64 ± 0.01^{c}
Cumulative energy intake (kcal)	405.9 ± 11.2^{a}	507.6 ± 12.9^{b}	453.7 ± 17.6^{ab}	450 ± 6.2^{a}

For diet consumption, mice were fed a low-fat diet (LF), a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. A one-way ANOVA was performed followed by a Tukey post-hoc test to compare diet consumption between groups. A different letter was attributed when the groups exhibit significant differences

the spectrophotometric method of Steegmans et al. using a commercial kit (K-FRUGL, Megazyme, Wicklow, Ireland)[16]. The contents in total/soluble/insoluble DF were determined in ITF supplements as previously described [15]. Of note, considering the proportion of the ITF supplements and cellulose incorporated in the different experimental diets, they are not matched in terms of total/soluble/insoluble DF. From a previous study [15], we can conclude that the degree of polymerisation of fructans was higher after steam cooking (13.5 versus 11.7). The proportion of soluble DF was much higher than insoluble DF (more than 70% versus 8%) and this proportion was not affected by the steam.

Mice were fed ad libitum for 6 weeks. At the 3rd week, a 24 h feces collection was performed. After 6 weeks, mice were fasted for 6 h and then anesthetized using isoflurane gas (Abbot, Ottignies, Belgium). Blood from cava vein was harvested in EDTA tubes. Portal blood was also collected and directly flushed within tubes containing dipeptidyl peptidase 4 inhibitor (Millipore, St Charles, MO, USA). Plasma was immediately collected after centrifugation (12 000 × *g* for 3 min) and stored at − 80 °C for biochemical analysis. Mice were necropsied after cervical dislocation. Liver, adipose tissues (epididymal, visceral, and subcutaneous), gastrocnemius muscles, cecal content and intestinal tissues were dissected and immersed in liquid nitrogen before storage at − 80 °C.

Metabolic measurements

Blood glucose levels were determined, after 6 h of fasting, using a glucose meter (Roche Diagnostics) on 3.5 µl of blood

collected from the tail vein. Plasma triglycerides, cholesterol and free fatty acid concentrations were measured using kits coupling enzymatic reaction and spectrophotometric detection of reaction end-products (Diasys Diagnostic and Systems, Holzheim, Germany). Plasma inflammatory mediators and portal concentrations of active glucagon-like peptide 1 (GLP-1) and total peptide YY (PYY) were determined using the Meso Scale Discovery (MSD) U-PLEX assay (Rockville, MD, USA) following the manufacturer's instructions. Analyses were performed using a QuickPlex SQ 120 instrument (MSD) and DISCOVERY WORKBENCH® 4.0 software (MSD, Rockville, MD, USA). The Dipeptidyl peptidase 4 (DPP4) activity was evaluated in the serum of the portal vein following a procedure previously described [17]. Lipid content was measured in the liver, gastrocnemius muscle and feces after extraction with chloroform–methanol according to the Folch method. Briefly, 100 mg of tissue (liver, muscle) or lyophilised feces were homogenised in 2 ml of chloroform: methanol (2:1). The chloroform phase was evaporated under nitrogen flux and the dried residue was weighted and solubilised in 1.5 ml of isopropanol. Triglyceride and cholesterol concentrations were measured using a kit coupling an enzymatic reaction and spectrophotometric detection of the final product (Diasys Diagnostic and System, Holzheim, Germany). For hepatic bile acids (BA) extraction, 50 to 100 mg of liver were homogenized at room temperature in 1 ml of 75% ethanol using a TissueLyser device (Qiagen). Homogenates were incubated 2 h at 50 °C and then centrifuged at 6,000 g for 10 min at 4 °C. Supernatants containing BA were collected. The assay for total BA concentration was performed as follows: 12 µL of hepatic BA samples or serum from portal vein were used for BA detection, using a colorimetric total Bile Acid Assay kit (Diazyme Laboratories, Inc., CA, USA), according to the manufacturer's instructions.

Histological analysis

In the ileum, the crypt depth and villus length were measured after hematoxylin/eosin staining. The sections were digitized (Leica SCN400, Leica Microsystems, Germany), and the images were captured using the Leica Image Viewer Software (Version 4.0.4). Crypt depth and villus length measurements were made on sections (at least 10 measurements per animal) by using Aperio ImageScope software.

RNA Extraction and real-time quantitative PCR

Total RNA was isolated from tissues using the TriPure isolation reagent kit (Roche Diagnostics, Penzberg, Germany). Complementary DNA was prepared by reverse transcription of 1 µg total RNA using the Kit Reverse Transcription System (Promega,Madison,WI). Real-time polymerase chain reaction (PCR) was performed with a CFX96 Touch Real-Time PCR Detection System and CFX manager 3.1 software (BioRad, Hercules, California, USA) using GoTaq qPCR Master Mix (Promega, WI, USA) for detection, according to the manufacturer's instructions. All samples were run in duplicate in a single 96-well reaction plate, and data were analyzed according to the $2^{-\Delta\Delta CT}$ method. The purity of the amplified product was verified by analyzing the melting curve performed at the end of amplification. The ribosomal protein L19 (RPL19) gene was chosen as a reference gene.

DNA Extraction and 16S rRNA Gene Sequencing

Genomic DNA was extracted from cecal content using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany), including a bead-beating step. Amplicon sequencing of the microbiome was done at the University of Minnesota Genomics Center. Briefly, the V5-V6 region of the 16S rRNA gene was PCR-enriched using the primer pair V5F_Nextera (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG**RGGATTAGATACCC**) and V6R_Nextera (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG**CGACRRCCATGCANCACCT**) in a 25 µl PCR reaction containing 5 µl of template DNA, 5 µl of 2X HotStar PCR master mix, 500 nM of final concentration of primers and 0.025 U/µl of HostStar Taq + polymerase (QIAGEN). PCR-enrichment reactions were conducted as follow, an initial denaturation step at 95 °C for 5 min followed by 25 cycles of denaturation (20 s at 98 °C), annealing (15 s at 55 °C), and elongation (1 min at 72 °C), and a final elongation step (5 min at 72 °C). Next, the PCR-enriched samples were diluted 1:100 in water for input into library tailing PCR. The PCR reaction was analogous to the one conducted for enrichment except with a KAPA HiFi Hot Start Polymerase concentration of 0.25 U/ µl, while the cycling conditions used were as follows, initial denaturation at 95 °C for 5 min followed by 10 cycles of denaturation (20 s at 98 °C), annealing (15 s at 55 °C), and elongation (1 min at 72 °C), and a final elongation step (5 min at 72 °C). The primers used for tailing are the following: F-indexing primer AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTC and R-indexing primer CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGG GCTCGG, where [i5] and [i7] refer to the index sequence codes used by Illumina. The resulting 10 µl indexing PCR reactions were normalized using a Sequal-Prep normalization plate according to the manufacturer's instructions (Life Technologies). 20 µl of each normalized sample was pooled into a trough, and a SpeedVac was used to concentrate the sample pool down to 100 µl. The pool was then cleaned using 1X AMPureXP beads and eluted in 25 µl of nuclease-free water. The final pool was quantitated by QUBIT (Life Technologies) and checked on a Bioanalyzer High-Sensitivity DNA Chip (Agilent Technologies)

to ensure correct amplicon size. The final pool was then normalized to 2 nM, denatured with NaOH, diluted to 8 pM in Illumina's HT1 buffer, spiked with 20% PhiX, and heat denatured at 96 °C for 2 min immediately prior to loading. A MiSeq 600 cycle v3 kit was used to sequence the pool.

Sequences data were demultiplexed at the Genomics Center platform from the University of Minnesota. For bioinformatics analysis, the Galaxy-supported pipeline FROGS (Find, Rapidly, Otus with Galaxy Solution) was used in order to produce abundance tables of Operational Taxonomic Units (OTUs) and their taxonomic affiliation [18]. For preprocessing step: reads are merged with VSEARCH, dereplicated, and filtered according to their length, mismatches in primers, and N content. A Swarm clustering with denoising and an agglomeration distance of 1 was then applied [19], allowing the creation of 108 143 clusters. FROGS chimera detection relies on VSEARCH with de novo UCHIME method [20, 21]. Chimera detection was performed, allowing the deletion of 73% of clusters and 13% of sequences. A filter for PhiX contaminant followed this step and for OTU abundance (minimum proportion/number of sequences to keep OTU was 0.00005%). This step deleted 98.5% of OTU and 1.7% of sequences to finally obtain 436 OTU. These 436 OTU were then affiliated using the SILVA database v138 for 16S data (pintail score 100) [22].

Alpha diversity indexes and beta diversity indexes were then evaluated using the FROGSSTAT Phyloseq stool. For alpha-diversity, the original table without normalization was used whereas data were normalized before the analysis of beta-diversity and phylotypes. PCoA plot of the beta-diversity indexes were visualized using Rstudio software, and a PERMANOVA test was performed. The phylotypes were computed as percent proportions based on the total number of sequences in each sample. Taxa significantly regulated by compounds (inulin or scorzonera) were identified using a Kruskal–Wallis test, followed by a Dunn's test in R software version 3.5.1. The p-value of the Kruskal–Wallis was then adjusted (q-value) to control for the false discovery rate (FDR) for multiple tests according to the Benjamini and Hochberg procedure [23].

Statistical analyses

Results are shown as mean ± SEM. Normal distribution was assessed using a Shapiro–Wilk normality test on GraphPad Prism software version 8.4.2. For the normal distribution, statistical analysis was performed by one-way analysis of variance (ANOVA) followed by a post hoc Tukey's multiple comparison tests. For others, a Kruskal–Wallis test was applied, followed by a Dunn's multiple comparisons test using GraphPad Prism 8.4.2. The results were considered statistically significant if $p < 0.05$. Data with different superscript letters were significantly different ($p < 0.05$) according to the post hoc statistical analysis.

Results

Influence of purified inulin and scorzonera extract on metabolic disorders related to obesity

The consumption of the HF diet containing 18% of cellulose (and therefore with 41.4% kcal coming from fat) induced obesity and fat mass expansion after 6 weeks of dietary treatment (Fig. 1); the body weight gain being doubled compared to LF group ($p < 0.05$ Student t-test). We investigated in HF-fed mice whether the supplementation of purified inulin (Inu) or fructans from a lyophilized preparation of scorzonera (Sco) could differentially impact the metabolic disorders associated with obesity. Throughout the experiment, all mice fed a HF gained body weight in a similar way compared to LF group (Fig. 1A–B). After 6 weeks, the cumulative food intake was lower in the Inu and Sco groups compared to both LF and HF (Fig. 1C). However, the total energy intake was not significantly affected (Fig. 1D). Feed efficiency, i.e. weight gain divided by calories consumed during the whole treatment, was higher with both sources of ITF supplementation but this effect did not reach significance (Fig. 1E). Considering free sugars present in the ITF extracts (Table 1), we calculated a lower intake of saccharose in Inu and Sco groups *versus* HF whereas the intake of free fructose and glucose were higher in ITF treated groups. Importantly, the content of fructose was higher in scorzonera product *versus* purified inulin extract, leading to a significant difference in the intake of fructose in Sco group compared to Inu group (approximately a 2.5-fold increase). The adipose tissue masses (subcutaneous, epididymal and visceral fat) were similar between the three HF groups (Fig. 1F–H).

Previous studies have shown in different models of obesity that ITF could regulate satietogenic gut peptides and lessen glycemia and triglyceridemia [5, 24]. In the present study, fasted hyperglycemia induced by 6-weeks treatment of HF diet was not influenced by both ITF supplementations (Table 2). Furthermore, inflammatory mediators, gut hormones controlling appetite and glycemia such as GLP-1 and PYY as well as enzyme DPP4 involved in their regulation [25, 26], were not significantly affected by ITF supplementation (Table 2). Surprisingly, Inu and Sco increased the levels of plasma triglycerides, compared to HF group without affecting the HF-induced higher cholesterolemia and plasma non-esterified fatty acids (NEFA) (Table 2).

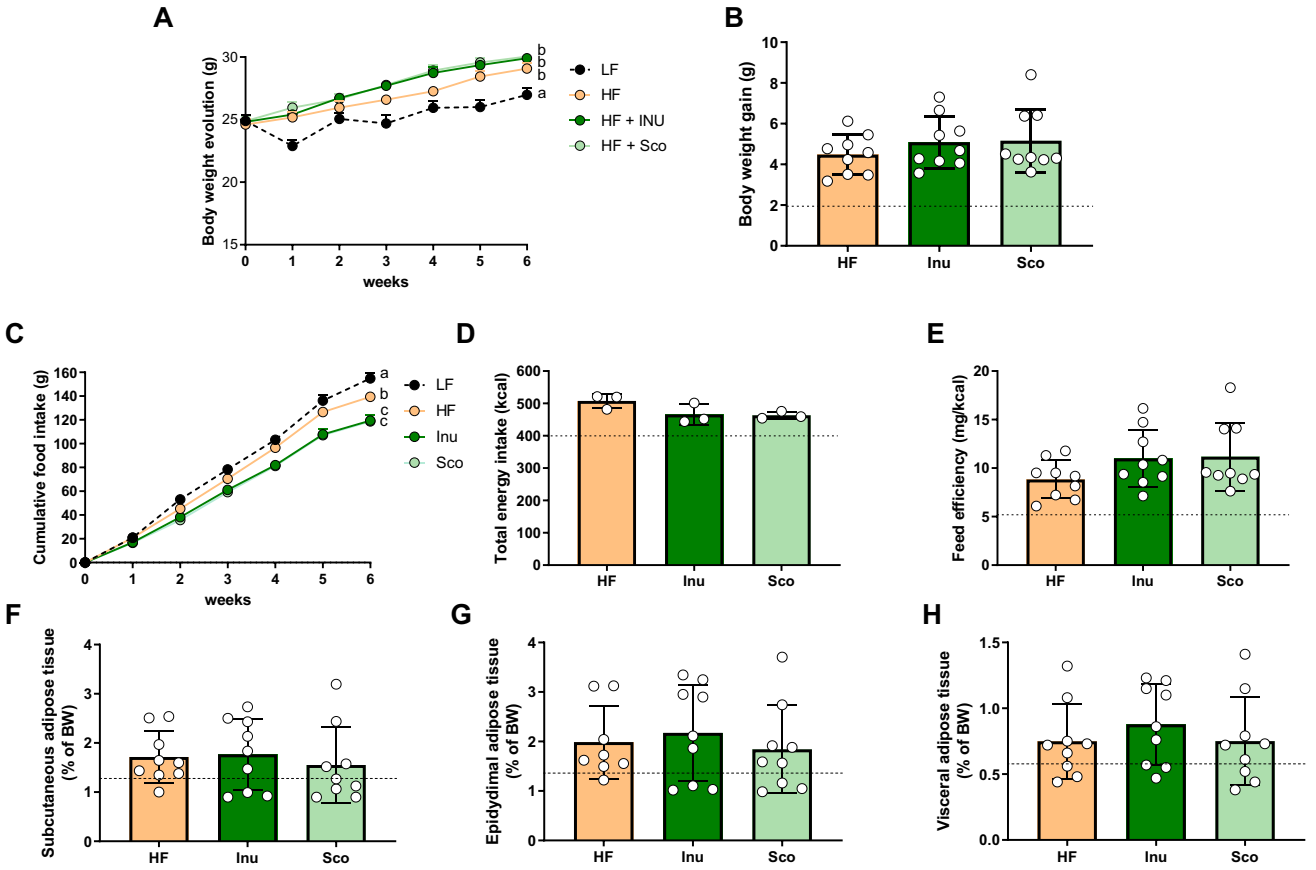

Fig. 1 Effects of purified inulin and lyophilized scorzonera on body weight gain, food intake and fat mass. Body weight evolution all along the experiment (**A**), body weight gain at the end of the experiment (**B**), cumulative food intake (**C**), total energy intake (**D**), feed efficiency (**E**), weight of adipose tissues (**F–H**) of mice fed a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. Data are expressed as mean ± SEM. The black dotted line represents the mean obtained for the control group of mice fed with a low-fat diet. For (**A**) and (**C**), a two-way ANOVA was performed and a Tukey post-hoc test was then applied for comparison between groups. For other parameters, a one-way ANOVA followed by a Tukey test or a Kruskal–Wallis test completed with a Dunn's test was applied, according to the normal distribution. A different letter (a,b or c) was attributed when the groups exhibit significant differences

Purified inulin and scorzonera extract differentially modulate lipid metabolism in the liver

We evaluated the influence of both ITF products on lipid metabolism. First, we observed lipid accumulation in *gastrocnemius* muscles in the Sco group (Supplemental Fig. 1A). We then focused on the regulation of lipid metabolism occurring in the liver after supplementation with Inu or Sco. Inulin significantly increased the liver weight whereas only a similar tendency was found in Sco group (Fig. 2A). This effect was not associated with an increased lipid accumulation in the liver (Fig. 2B–C). On the contrary, supplementation with purified inulin significantly reduced total hepatic lipids and triglycerides, whereas only a tendency to limit hepatic lipids accumulation was observed Sco group *versus* HF group. Hepatic cholesterol level remained similar between the three HF-fed groups (Fig. 2D). The expression of key genes involved in the regulation of lipid metabolism in the liver revealed that inulin extract reduced the mRNA level of *Cd36* (cluster of differentiation 36) and C*pt1a* (carnitine palmitoyltransferase 1 alpha), two markers involved in the transport of fatty acids (FA) into the cells and mitochondria, respectively (Fig. 2E). The expression of several genes involved in lipogenesis and FA oxidation remained unchanged between the three HF groups after 6 weeks of treatment. However, the mRNA level of *Apob* (apolipoprotein B), involved in the very low-density lipoprotein (VLDL) synthesis, significantly decreased in both Inu and Sco groups, compared to HF (Fig. 2E).

Although we did not detect significant differences in total bile acids (BA) in the serum among the three HF groups (Table 2), both ITF sources increased the concentration of hepatic BA (Fig. 2F). This last effect reached significance only with scorzonera extract (Fig. 2F). Therefore,

Table 2 Plasma parameters

	LF	HF	Inu	Sco
Fasted glycemia (mg/dl)	129.1 ± 7.7[a]	166.6 ± 8.3[b]	173.4 ± 8.8[b]	170.0 ± 13.1[b]
GLP-1 (pM)	0.42 ± 0.08	0.57 ± 0.10	0.57 ± 0.10	0.45 ± 0.05
PYY (pg/ml)	30.7 ± 2.2	29.1 ± 3.2	38.5 ± 2.9	37.4 ± 3.9
DPP4 activity (mU/ml)	5.33 ± 0.45[a]	6.60 ± 0.27[b]	5.62 ± 0.18[ab]	5.75 ± 0.36[ab]
IL-6 (pg/ml)	166.3 ± 61.4	209.5 ± 58.5	159.3 ± 37.1	250.2 ± 48.3
IL-10 (pg/ml)	73.8 ± 5.5	74.5 ± 5.9	82.6 ± 3.6	91.1 ± 6.3
KC/GRO (pg/ml)	174.3 ± 14.9	241.9 ± 29.5	220.7 ± 32.7	231.6 ± 26.5
CCL2 (pg/ml)	78.9 ± 9.6	114.3 ± 14.3	103.9 ± 12.7	112.6 ± 11.6
TNFα (pg/ml)	41.1 ± 2.0[a]	51.1 ± 2.4[a,b]	53.7 ± 4.08[b]	60.2 ± 3.6[b]
Triglycerides (mM)	0.21 ± 0.03[a]	0.26 ± 0.03[a]	0.44 ± 0.05[b]	0.44 ± 0.05[b]
Cholesterol (mM)	2.05 ± 0.09[a]	2.49 ± 0.12[b]	2.35 ± 0.10[ab]	2.44 ± 0.06[b]
NEFA (mM)	0.34 ± 0.03	0.37 ± 0.03	0.33 ± 0.02	0.36 ± 0.02
Total bile acids (µM)	49.9 ± 7.2	53.0 ± 6.9	52.4 ± 7.9	57.4 ± 11.7

Mice were fed a low-fat diet (LF), a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. A one-way ANOVA was performed followed by a Tukey post-hoc test to compare diet consumption between groups. A different letter (a or b) was attributed when the groups exhibit significant differences. *CCL2* C–C motif chemokine ligand 2; *DPP4* Dipeptidyl peptidase 4; *GLP-1* Glucagon-like peptide-1; *IL* Interleukin; *NEFA* Non-esterified fatty acids; *PYY* Peptide YY; TNFα, tumor necrosis factor-alpha

we explored the mRNA expression of genes involved in the hepatic BA pathway (Fig. 2G). Whereas markers playing a role in BA uptake and export remained similar between all HF groups, Inu increased the mRNA level of *Nr0b2* (also known as SHP) and decreased the expression of *Cyp7a1* mRNA (Cytochrome P450 family 1 subfamily A member 1), both being regulators of BA synthesis from cholesterol. This last result suggested that Inu supplementation could decrease hepatic BA synthesis through the classical pathway without affecting total level of BA in the serum.

Purified inulin and scorzonera extract differentially modulate the gut physiology

Some of data presented before suggested an impact of inulin on fecal lipid excretion [27]. We observed that Sco significantly reduced the total feces weight collected during 24 h without affecting the number of excreted pellets (Fig. 3A–B). The measurement of fecal lipids content showed that Sco increased the fecal lipids and cholesterol (but not triglycerides) excretion (per day and per mouse), compared to other HF groups (Fig. 3C–E). Regarding the intestinal physiology, we found an increase in crypts depth, without modification of villus length, was observed in Sco group compared to others (Fig. 3F). For the three markers of antimicrobial peptides investigated, we showed that Sco increased the ileal mRNA expression of *Reg3g, Defα, Pla2g2a*, whereas Inu only increased *Reg3g* (Fig. 3G); those effects were not observed in the colon tissue (Supplemental Fig. 2). However, we obtained contrasting data for markers involved in the gut barrier function (Fig. 3H). Sco significantly increased occludin mRNA expression (*Ocln*), but reduced the *Tjp1* mRNA level only in the ileal tissue (Fig. 3H; Supplemental Fig. 2). We then evaluated the expression of genes involved in immunity in the ileum and the colon (Fig. 3I, Supplemental Fig. 2). We found that Sco upregulated the mRNA levels of *Il1β* as well as two markers of macrophages recruitment (*Adgre1* known as F4/80 and *Ccl2* known as MCP-1) in the ileum compared to HF group. In the colon tissue, both fructan sources increased TNF-α expression (*Tnf*) in the same extent.

We assessed the intestinal BA pathway analysis and demonstrated that both Inu and Sco restored the mRNA levels of ileal bile acid transporter *(Slc10a2* also known as IBAT*)* upon a HF (Fig. 3J). However, we only detected a significant increase of fibroblast growth factor 15 *(Fgf15)* in the ileum of mice receiving purified inulin. Interestingly, Inu also significantly decreased G protein-coupled bile acid receptor 1 *(Gpbar1;* also known as TGR5) and increased nuclear receptor subfamily 1 group H member 4 (*Nr1h4;* also known as FXR), those receptors being involved in energy homeostasis, BA pool, insulin signaling or immune response [28].

Purified inulin and scorzonera extract are fermented at the same extend but differentially regulate the gut microbiota composition at the genus level

The expansion of cecal content was similar for Inu and Sco groups compared to HF (Fig. 4A). This effect was also observed for the cecal tissue (data not shown), suggesting that the gut fermentation occurred in a similar way within these two groups. We then analyzed the gut

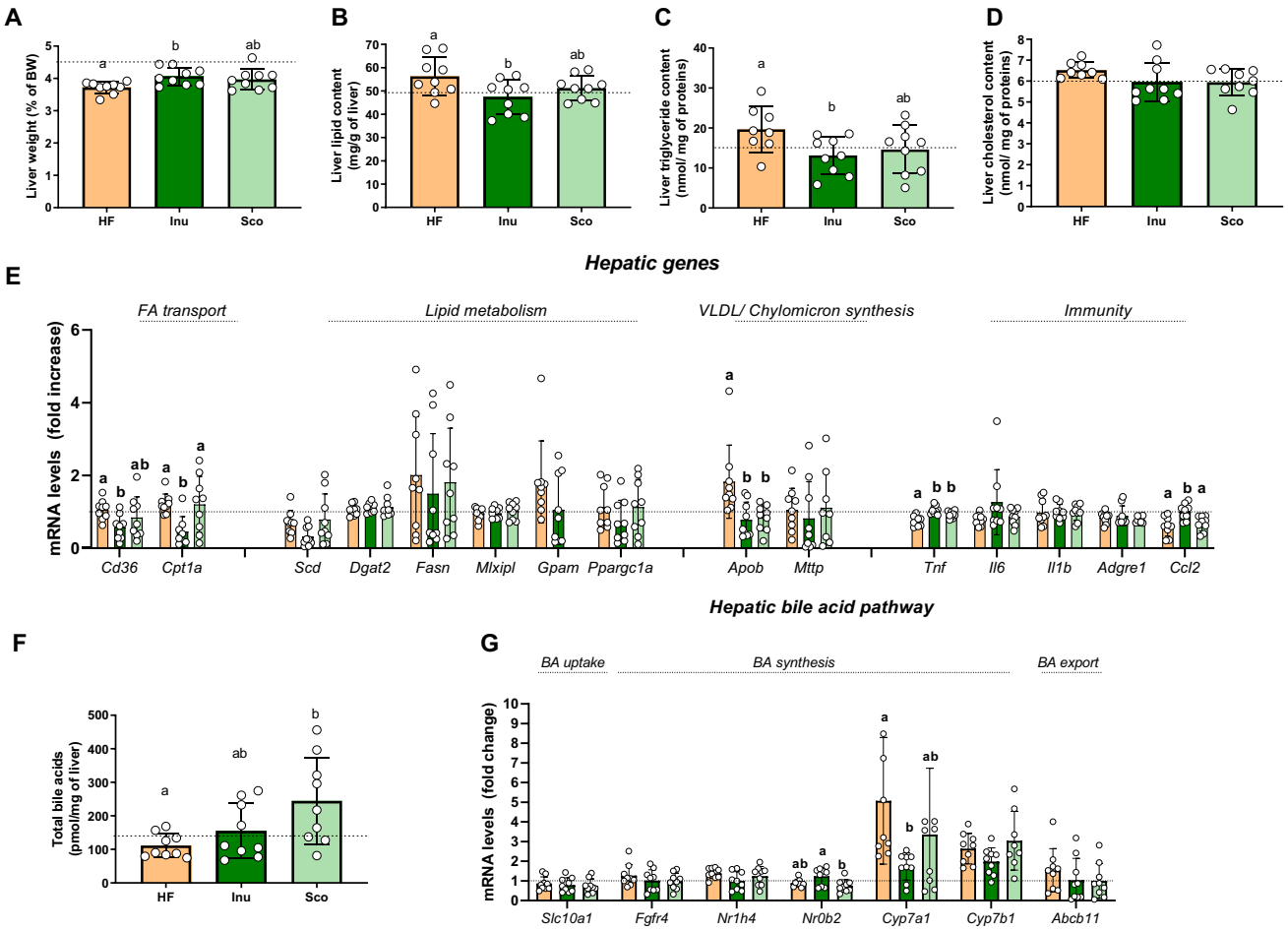

Fig. 2 Effects of purified inulin and lyophilized scorzonera on lipid metabolism in the liver. Liver mass (% of body weight, BW) (**A**), hepatic contents of lipids (**B**), triglycerides (**C**) and cholesterol, expression of hepatic genes involved lipid transport (**D**), synthesis and oxidation (**E**), hepatic content of total bile acids (BA) (**F**), expression of hepatic genes involved in BA uptake, synthesis and export (**G**) in mice fed a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. Data are expressed as mean ± SEM. The black dotted line represents the mean obtained for the control group of mice fed with a low-fat diet. A one-way ANOVA was performed, followed by a Tukey post-hoc test for comparison between groups. A different letter (a or b) was attributed when the groups exhibit significant differences

microbiota composition in the cecal content of mice, by using 16S rRNA gene Illumina sequencing. We first confirmed that both richness and evenness indexes used for evaluating the α-diversity of samples, decreased in HF versus LF group (Fig. 4B–F). Both Inu and Sco restored the different indexes during a HF diet, the effects being more significant in Sco group compared to Inu. Principal coordinate analysis (PcoA) of the Bray–Curtis distance showed a clear separation of the HF group compared to other groups (Fig. 4G, adonis permanova, $p < 0.001$ for treatment effect). Nevertheless, Inu and Sco samples are clearly separated from the ones included in LF or HF groups, but are clustered close together. At phyla level, we also observed similitudes between Inu and Sco groups (Fig. 4H, Table 3). We observed a similar increase of Actinobacteria, Bacteroidetes, Proteobacteria phyla (recently renamed Actinomycetota, Bacteroidota and Pseudomonadota, respectively) with Inu and Sco, as well as an important decrease of Firmicutes (recently renamed Bacillota) and Desulfobacterota compared to HF group. Even if we did not detect significant differences between Inu and Sco at the phylum level, the regulation of Cyanobacteria and Verrucomicrobia (recently renamed as Cyanobacteriota and Verrucomicrobiota, respectively) were only significant for Sco group compared to HF (Table 3). The visualization of barplots for the most abundant families detected in all samples showed a similar pattern between Inu and Sco samples, especially for *Bacteroidaceae, Bifidobacteriaceae* or *Desulfovibrionaceae* among others (Fig. 4I). More in details, the analysis of genera indicated several huge differences between HF versus both Inu and Sco groups (Fig. 4J and Table 3). At the genus level, more

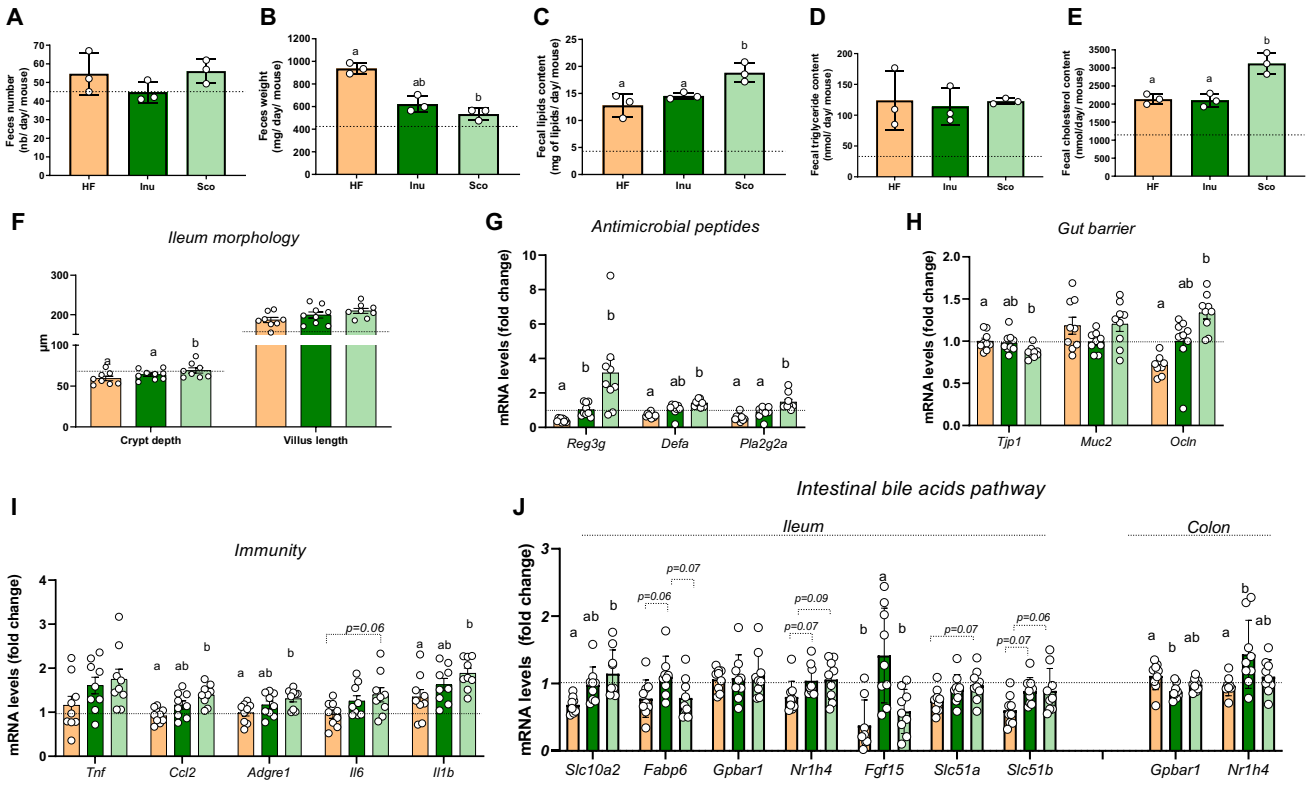

Fig. 3 Effects of purified inulin and lyophilized scorzonera on gut transit and intestinal physiology. Number of fecal pellets (**A**), feces weight (**B**), fecal contents of lipids (**C**), triglyceride content (**D**) and cholesterol content (**E**) in feces collected over 24 h from mice fed a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 3 weeks. Villus length and crypth depth in the ileum (**F**), expression of genes of antimicrobial peptides (**G**), gut barrier (**H**), immunity (**I**) and bile acid pathway (**J**) in the ileum or colon of mice fed a high-fat diet (HF) or a diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. Data are expressed as mean ± SEM. The black dotted line represents the mean obtained for the control group of mice fed with a low-fat diet. A one-way ANOVA followed by a Tukey test or a Kruskal–Wallis test completed with a Dunn's test was applied, according to the normal distribution. A different letter (a or b) was attributed when the groups exhibit significant differences

bacteria were significantly regulated after supplementation with scorzonera extract than purified inulin (Fig. 4J). Among them, the regulation in relative abundance of *Bifidobacterium spp., Butyricicoccus, Parasutterella, Peptococcus, Ruminococcus* and some other ones are similar in Inu and Sco groups *versus* HF group. We observed that the relatively high abundance of *Akkermansia* (8%) in the LF group dropped drastically in the HF group (reaching only 1%) and supplementation with both ITF partially restored its abundance (5% and 6%, respectively). In contrast, the level of members of *Desulfovibionaceae* observed both in the LF and HF groups (8% and 12%, respectively) were decreased at the same extent after supplementation with purified inulin or scorzonera extract (4% or 3%, respectively). The higher abundance of *Akkermansia* genus and of an unclassified members of *Prevotellaceae*, as well as the decrease in *Bacteroides* were only significant in Sco *versus* HF. In addition, the decreased abundance of *Alistipes* and *Blautia* by fructans were only observed in Inu, but not in Sco, compared to HF.

Correlation between gut microbiota changes and biological outcomes upon purified inulin and scorzonera extract supplementation

The Fig. 5 shows correlation analysis between the genera differently and significantly expressed between groups and the metabolic parameters significantly affected either by purified inulin or scorzonera extract supplementation in the high-fat diet. We observed many correlations between several genera and the cecal content mass expansion, signing the gut fermentation by fructans, independently of their source. Positive correlations were found between ileal expression of antimicrobial peptides (*Reg3g, Pla2ga2)* and of a tight junction protein (*Ocln*) with important genera such as *Akkermansia* and *Bifidobacterium*. Those markers of gut barrier were also positively correlated with the abundance of an unclassified member of *Prevotellaceae.* In addition, crypt depth was positively correlated with bifidobacteria but also with *Faecalibaculum*. Interestingly, steatosis (i.e. hepatic content of lipids and triglycerides) was

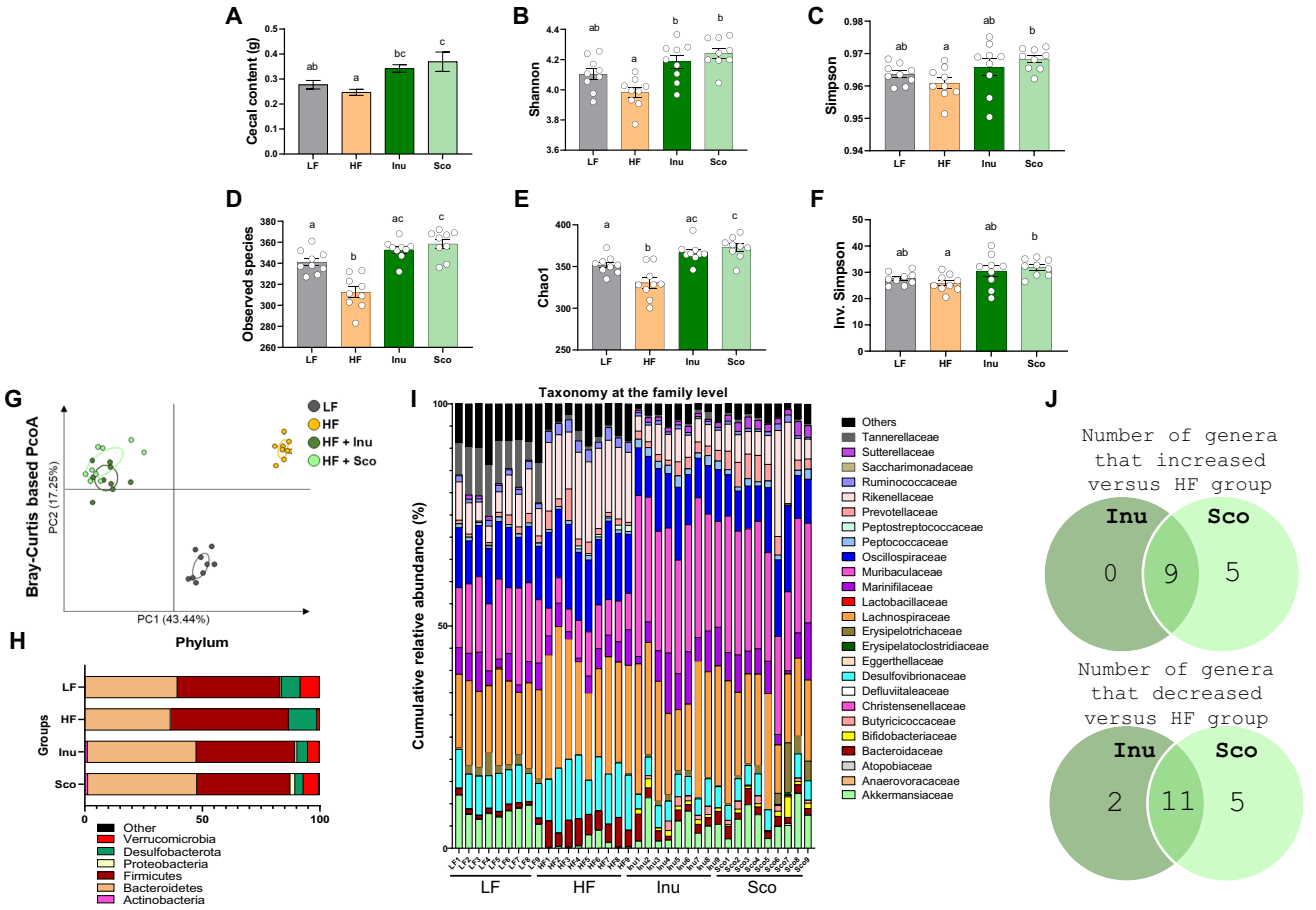

Fig. 4 Effects of purified inulin and lyophilized scorzonera on the overall gut microbiota composition. Weight of cecal content (**A**), α-diversity indices (**B–F**), β-diversity index through principal coordinates analysis (PCoA) of the Bray–Curtis distance (**G**) and barplots of relative abundance, at the phylum (**H**) or family (**I**) levels in mice fed a low-fat diet (LF), a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. Data are expressed as mean ± SEM. A one-way ANOVA was performed, followed by a Tukey post-hoc test for comparison between groups. A different letter (a, b or c) was attributed when the groups exhibit significant differences. Venn diagram showing the number of bacteria (at the genus level) significantly regulated by Inu and/or Sco versus HF group (**J**)

negatively related to *Bifidobacterium*. The gastrocnemius lipids accumulation reported in mice treated with scorzonera extract may be linked to a decrease in *Bacteroides*. Then, we decided to study the interconnections between the significant metabolic changes and genera significantly modified by the different diets by using HAIIA, a end-to-end statistical method for Hierarchical All-against-All discovery of significant relationships among data features with high power [29]. Correlations have been clustered depending on the correlation trend so that the genera that correlate with the same metabolic change (or metabolic changes that correlate with the same genus) are depicted together (Fig. 5B). HAllA identified several genera that correlated with different metabolic changes. In general, we observed a large number of cluster associations for gut parameters than for hepatic parameters. Notably, *Bifidobacterium* was found to present the most negative correlations of any bacteria with liver lipid contents and apolipoprotein B. This same genus clustered with other reported altered genera (*Peptococcus* and an unclassified member of *Desulfovibrionaceae*) but in opposite manner since they were found to be positively correlated with those parameters of liver steatosis. The same cluster of 3 genera were found also to correlate with expression of antimicrobial peptides (*Reg3g, Pla2ga2*) in the ileum. Of note, HAllA correlation analysis revealed that the change in crypt depth correlated only with *Faecalibaculum* but not with *bifidobacterium* as previously observed in the Fig. 5A. The second important cluster highlighted that *Akkermansia* together with *Olsenella*, *Parasutterella*, an unclassified member of *Prevotellaceae* and an unclassified member of *Gastranaerophilales* positively correlated with parameters related to gut physiology and gut barrier such (*Reg3g*, *Pla2ga2, Ocln*).

Table 3 Taxa regulated by purified inulin or scorzonera extract in the cecal content of HF-fed mice

	LF	HF	Inu	Sco	q-value	LF vs HF	HF vs Inu	HF vs Sco	Inu vs Sco
Phylum									
Actinobacteria	0.119 ± 0.026	0.046 ± 0.006	1.073 ± 0.146	1.252 ± 0.502	< 0.001	ns	< 0.001	0.003	ns
Bacteroidetes	39.19 ± 0.954	36.322 ± 1.016	46.262 ± 1.099	46.362 ± 1.695	< 0.001	ns	< 0.001	< 0.001	ns
Cyanobacteria	0.133 ± 0.037	0.066 ± 0.034	0.169 ± 0.047	0.374 ± 0.096	<0.01	ns	ns	<0.01	ns
Desulfobacterota	7.936 ± 0.267	11.959 ± 0.754	4.522 ± 0.282	3.6 ± 0.685	< 0.001	ns	< 0.001	< 0.001	ns
Firmicutes	43.796 ± 1.2	50.277 ± 1.184	42.02 ± 1.186	39.774 ± 1.83	< 0.001	ns	<0.01	< 0.001	ns
Proteobacteria	0.593 ± 0.098	0.071 ± 0.022	0.916 ± 0.105	1.955 ± 0.233	< 0.001	ns	<0.01	< 0.001	ns
Verrucomicrobia	8.16 ± 0.643	1.136 ± 0.477	4.971 ± 1.113	6.454 ± 1.112	< 0.001	< 0.001	ns	<0.01	ns
Genus									
Akkermansia	8.16 ± 0.643	1.136 ± 0.477	4.971 ± 1.113	6.454 ± 1.112	< 0.001	< 0.001	ns	<0.01	ns
Alistipes	5.928 ± 0.712	11.773 ± 0.612	4.86 ± 0.424	8.753 ± 2.38	<0.01	< 0.05	< 0.001	ns	ns
Anaerotruncus	0.1 ± 0.015	0.297 ± 0.078	0.009 ± 0.003	0.044 ± 0.011	< 0.001		< 0.001	<0.01	ns
Bacteroides	1.046 ± 0.09	4.921 ± 0.431	2.462 ± 0.493	1.992 ± 0.26	< 0.001	< 0.001	ns	< 0.05	ns
Bifidobacterium	0.001 ± 0.001	0.001 ± 0.001	1.044 ± 0.143	1.146 ± 0.451	< 0.001	ns	< 0.001	<0.01	ns
Blautia	1.33 ± 0.267	4.249 ± 0.845	0.481 ± 0.177	1.705 ± 0.659	<0.01	ns	< 0.001	ns	ns
Butyricicoccus	0.014 ± 0.005	0.004 ± 0.002	1.001 ± 0.288	0.876 ± 0.207	< 0.001	ns	< 0.001	< 0.001	ns
Colidextribacter	4.337 ± 0.241	6.431 ± 0.364	3.297 ± 0.107	3.24 ± 0.4	< 0.001	ns	< 0.001	< 0.001	ns
Eisenbergiella	0.238 ± 0.069	0.019 ± 0.004	0.001 ± 0.001	0.002 ± 0.001	< 0.001	ns	0.075	< 0.05	ns
Faecalibaculum	1.223 ± 0.27	0.028 ± 0.012	0.139 ± 0.043	2.126 ± 1.105	< 0.001	< 0.001	ns	<0.01	ns
Lachnoclostridium	0.111 ± 0.036	0.347 ± 0.039	0.761 ± 0.163	0.457 ± 0.138	<0.01	ns	ns	ns	ns
Olsenella	0.101 ± 0.024	0.001 ± 0.001	0.003 ± 0.001	0.077 ± 0.05	< 0.001	< 0.001	ns	ns	ns
Parabacteroides	8.788 ± 0.84	0.65 ± 0.214	0.455 ± 0.144	0.325 ± 0.064	< 0.001	<0.01	ns	ns	ns
Parasutterella	0.45 ± 0.094	0.007 ± 0.002	0.847 ± 0.096	1.913 ± 0.229	< 0.001	ns	<0.01	< 0.001	ns
Peptococcus	0.078 ± 0.01	0.22 ± 0.021	0.069 ± 0.014	0.026 ± 0.007	< 0.001	ns	<0.01	< 0.001	ns
Roseburia	0.024 ± 0.005	0.237 ± 0.079	0.433 ± 0.159	0.489 ± 0.181	< 0.001	< 0.05	ns	ns	ns
Ruminococcus	0.033 ± 0.005	0.012 ± 0.006	0.122 ± 0.02	0.161 ± 0.065	< 0.001	ns	< 0.001	<0.01	ns
Turicibacter	0 ± 0	0.058 ± 0.027	0 ± 0	0 ± 0	<0.01	<0.01	<0.01	<0.01	ns
Tuzzerella	0.589 ± 0.056	0.728 ± 0.033	0.032 ± 0.009	0.06 ± 0.037	< 0.001	ns	< 0.001	< 0.001	ns
Uncl. Erysipelotrichaceae	0.673 ± 0.195	0.009 ± 0.004	0.58 ± 0.12	0.686 ± 0.204	0.001	0.002	0.004	0.005	ns
Uncl. Oscillospiraceae	1.202 ± 0.111	0.537 ± 0.038	2.866 ± 0.474	2.126 ± 0.366	< 0.001	ns	< 0.001	< 0.001	ns
Uncl. Peptostreptococcaceae	0.026 ± 0.014	0.488 ± 0.161	0 ± 0	0.001 ± 0.001	0.022	ns	0.012	0.036	ns
Uncl. Prevotellaceae	0.293 ± 0.043	0.091 ± 0.025	0.203 ± 0.036	2.768 ± 0.468	< 0.001	0.05	ns	< 0.001	0.005
Uncl. Rikenellaceae	0.827 ± 0.245	4.497 ± 0.482	0.329 ± 0.047	0.223 ± 0.115	< 0.001	ns	0.002	< 0.001	ns
unknown_ Bacilli	0.024 ± 0.009	0 ± 0	0.111 ± 0.025	0.098 ± 0.043	0.001	ns	< 0.001	0.002	ns
unknown_ Clostridia	9.278 ± 0.726	5.864 ± 0.725	2.03 ± 0.346	1.731 ± 0.335	< 0.001	ns	ns	0.025	ns
unknown_ Desulfovibrionaceae	7.82 ± 0.273	11.781 ± 0.767	4.421 ± 0.284	3.354 ± 0.645	< 0.001	ns	< 0.001	< 0.001	ns
unknown_ Gastranaerophilales	0.133 ± 0.037	0.066 ± 0.034	0.169 ± 0.047	0.374 ± 0.096	0.008	ns	ns	0.004	ns
unknown_ Lachnospiraceae	5.551 ± 0.582	7.516 ± 0.51	6.825 ± 0.514	5.058 ± 0.292	0.009	0.043	ns	0.008	ns
unknown_ Muribaculaceae	16.434 ± 0.847	7.864 ± 0.594	28.342 ± 1.215	25.254 ± 1.333	< 0.001	ns	< 0.001	< 0.001	ns
unknown_ Oscillospiraceae	5.086 ± 0.352	7.377 ± 0.267	3.923 ± 0.291	5.16 ± 1.048	0.006	ns	< 0.001	0.01	ns
unknown_ Oscillospirales	0.282 ± 0.036	0.212 ± 0.034	0.522 ± 0.101	0.717 ± 0.1	0.001	ns	ns	< 0.001	ns
unknown_ Peptococcaceae	0.698 ± 0.051	0.524 ± 0.047	1.776 ± 0.139	1.173 ± 0.194	0.005	ns	< 0.001	0.022	ns
unknown_ Rhodospirillales	0.143 ± 0.03	0.064 ± 0.023	0.069 ± 0.013	0.042 ± 0.013	0.020	ns	ns	ns	ns
unknown_ Ruminococcaceae	0.967 ± 0.082	2.053 ± 0.174	0.457 ± 0.036	0.591 ± 0.072	< 0.001	ns	< 0.001	< 0.001	ns

Relative abundance of taxa significantly regulated by inulin or scorzonera in the cecal content of mice fed a low-fat diet (LF), a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. Results are expressed as mean for the percentage of relative abundance ± SEM. Significantly affected taxa by inulin or scorzonera were identified using a Kruskal–Wallis ANOVA performed in R. p-value was adjusted (q-value, significant if $q < 0.05$) to control for the false discovery rate (FDR correction) for multiple testing. ns = not significant. Multiples comparisons were then assessed using a Dunn's posthoc test in R and when significant, the p-value is reported

Discussion

In this study, we demonstrated that two types of ITF, one given as natural source (scorzonera) and one given as a purified extract from chicory root at the same dose, may have some similar expected effects on gut microbiome modulation (increase in bifidobacteria), but can also be distinct in terms of specific gut bacterial changes, as well as on

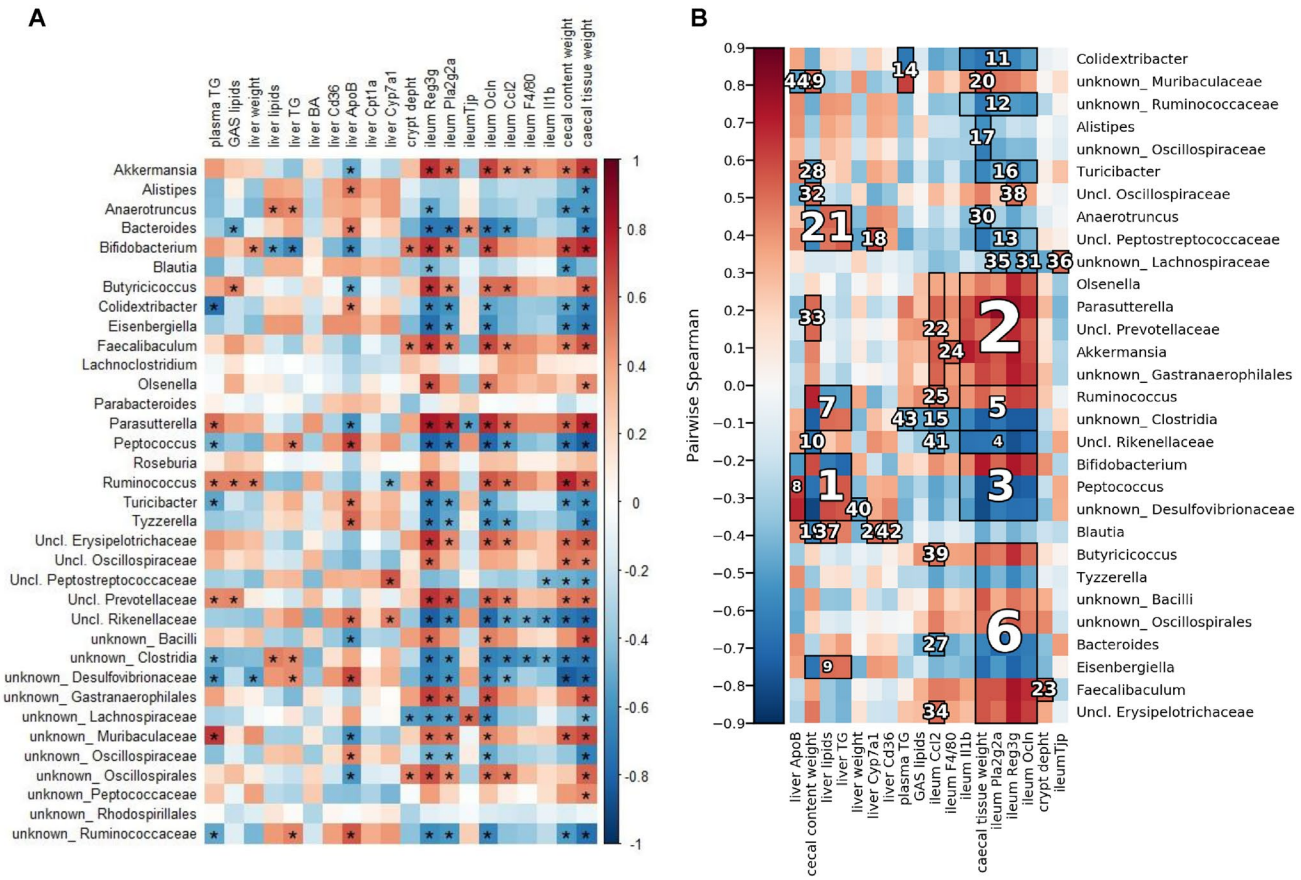

Fig. 5 Correlations analysis between genera and metabolic parameters significantly modified by the different diets. Both heatmaps considered data obtained from mice fed a high-fat diet (HF) or a high-fat diet enriched with purified inulin (Inu) or lyophilized scorzonera (Sco) for 6 weeks. Heatmap of Spearman's correlations (**A**). HAllA diagram presenting 44 strongest association (**B**). The red color indicated positive correlation values, while blue color negative ones.. *q < 0,05, FDR correction

biological outcomes in HF-diet fed mice. Mice developed the obese phenotype (higher body weight gain, fat mass expansion, higher glycemia and higher cholesterolemia versus LF diet) with the HF diet containing non-fermentable cellulose (18%) already after 6 weeks of dietary treatment, as previously shown with a HF diet providing 45% kcal from fat [30, 31]. However, none of the ITF sources influence the body weight or fat mass, but the effects of fructans on metabolic parameters vary according to their source. In fact, the total cumulative food intake in gram is decreased by both fructan supplementations in the high-fat diet (Inu and Sco) but this effect appears after 4 weeks of dietary intervention. Therefore, the consequence on body weight and fat mass was not significant 2 weeks later. Maybe increasing the length of the treatment would have led to a significant effect on adiposity and body weight. Indeed, we and others have shown an effect of fructans that decreased adiposity and body weight after 7 weeks of inulin supplementation in HF-induced obesity models (for review, see [32]). In addition, our data show that the supplementation of the high-fat diet with purified inulin or scorzonera extract increased the levels of plasma triglycerides, compared to HF group without affecting plasma cholesterol and non-esterified fatty acids (Table 2). The accumulation of lipids observed in the muscle of mice receiving scorzonera extract is not illogical, in view of parallel increase in serum triglycerides suggesting a higher fatty acids availability for peripheral organs like muscle. When detailing liver lipids, we did not see any effect with scorzonera extract but a decrease with inulin that cannot be explained by higher excretion of triglycerides in the feces. Quite specific effect of scorzonera was observed on cholesterol excretion in the feces that was not translated into changes in cholesterol levels in the serum or the liver as compared to HF diet. Concerning BA turnover, we observed an increase in liver BA by scorzonera supplementation independently of a regulation of genes coding for intestinal BA uptake, export or synthesis.

We observed that only ITF from chicory exerted beneficial effects on liver lipid accumulation and lowered the hepatic lipids accumulation during a HF diet, this being

associated with a decreased expression of *Cd36* mRNA involved in fatty acid transport into the cells. This is consistent with our previous observations in HF-fed mice [27]. Purified inulin regulated the expression of genes involved in BA synthesis (reduced *CYP7a1* expression) in the liver, and increased markers of BA transport in the ileum. An increase in FGF15 expression in the ileum of mice fed purified chicory ITF could be of interest, knowing that lack of FGF15, a BA-induced ileum-derived enterokine that governs BA homeostasis, results in increased hepatic steatosis and in the development of endoplasmic reticulum stress in the liver of mice fed a high fat diet [33]. We observed a higher level of total BA in the liver of Sco mice only, compared to HF. We also detected an increased intramuscular lipids accumulation and a higher amount of fecal lipids content in this group supporting the fact that lipid metabolism is differentially modulated by purified inulin from chicory root *versus* lyophilized scorzonera extract. We also noticed that despite a reduction of ApoB mRNA in the liver of both groups of mice supplemented with ITF, there was an increase in plasma triglycerides in both supplemented groups. We hypothesized that the presence of low level of free fructose and glucose in the ITF sources (inulin and scorzonera) could partially explain this observation [34].

Both sources of ITF also exerted differential effects on the intestine. None of them could influence the expression of genes reflecting the production of gut hormones involved in appetite or glycemia regulation (data not shown), despite an observation of a decrease in food intake in both groups supplemented with ITF compared to HF group. Our previous data have shown that oligofructose supplementation stimulated mucus production and expression of Muc2 in the intestinal tissue of mice fed a HF diet [35], and other authors have also shown the beneficial effect of inulin on mucus deterioration in colitis model [36, 37]. The improvement of gut barrier function by supplement of inulin or oligofructose associated to induction of antimicrobial peptides, has already been observed in mice fed with a HF diet or a "western style" diet for 6 to 12 weeks [38, 39]. Here, we report the positive effects of native inulin when given as dietary source on gut homeostasis. Indeed, when ITF were administrated as powdered scorzonera vegetables (salsify), they mostly induced beneficial effects on the intestines (upregulation of markers involved in the gut barrier function and antimicrobial peptides, increased crypts depth size) after 6 weeks of dietary supplementation, those effects being not observed in animals fed a in the high-fat diet supplemented with purified inulin from chicory root. Curiously, the effects were mainly observed in the ileum tissue compared to the colonic tissue. The expression of genes reflecting the activation of the gut immune system (cytokines and markers of macrophages recruitment) was higher in Sco group versus HF. Such an effect has already been described for other nutrients with prebiotic effect. For example, the supplementation with spirulina in aged mice reduced severe liver inflammation which was coordinated with an increase innate immunity in the gut [40].

The potential mechanisms underlying the differential effects of inulin sources can be attributed to a huge variety of metabolites produced by the gut microbiome [41]. The bacterial genera increased in the cecal content of Inu and Sco groups, such as *Bifidobacterium* spp., *Butyricicoccus, Parasutterella, Peptococcus* and *Ruminococcus,* are notably short-chain fatty acid (SCFA)-producers. However, measuring SCFA in the fecal matter does not necessarily reflect a higher production by bacteria since those SCFA can be absorbed and metabolized [42, 43]. In our study, the comparison of scorzonera and chicory inulin effects on the gut microbiota composition led to several similarities on the overall composition. At the phyla level, abundance of Actinobacteria, Bacteroidetes, Proteobacteria phyla (recently renamed Actinomycetota, Bacteroidota and Pseudomonadota, respectively) increased with sources of ITF whereas Firmicutes (recently renamed Bacillota) and Desulfobacterota decreased. It is interesting to note that the high level of bacteria belonging to *Desulfovibrionaceae* observed in the LF and HF groups dropped at the same extent after ITF supplementations because many studies have reported that those sulfate-reducing bacteria were positively correlated with obesity and metabolic syndrome phenotype [44]. However, some differences can be observed with a more detailed analysis based on the regulation of taxa. For instance, Sco group exhibited a greater increase of richness indexes (observed species, chao1) than Inu group. However, it is quite interesting to observe that the two sources of fructans can affect differently some genera such as *Akkermansia, Alistipes, Bacteroides, Blautia*, or *Faecalibaculum.* These differences are quite interesting since the regulation of *Akkermansia*, *Alistipes, Bacteroides* and *Blautia* are among key markers determining the efficacy of metabolic response upon inulin in mice inoculated with stools from human donors with obesity [14].

Compared to HF group, Sco increased *Akkermansia* and *Faecalibaculum,* and decreased *Bacteroides,* an effect not observed with inulin from chicory. Among the correlation observed, the positive association between *Akkermansia* and several markers of gut barrier function (antimicrobial peptides, infiltrating immune cells, tight junction proteins) are all in line with several studies having shown that *Akkermansia muciniphila* supplementation protect against HF diet induced gut barrier dysfunction and metabolic disorders [45–47]. In addition, the abundance of *Blautia* was only reduced with inulin compared to HF. To note, the only taxa significantly different between Inu and Sco groups was an unclassified *Prevotellaceae,* largely present in Sco group. Among *Prevotellaceae* family, the genus *Prevotella* is known

to be associated with fibre intake [48]. This is quite interesting to observe that the abundance of unclassified members of *Prevotellaceae* can be increased with scorzonera but not with purified inulin. In addition, the increase of this unclassified *Prevotellaceae* correlated with the increase of some markers observed in Sco group (cecal content, intramuscular lipids, gut barrier function and antimicrobial peptides). In a previous study, we determined the DF profile (total DF, low- and high-molecular-weight soluble DF, and insoluble DF) and the degree of polymerisation (DP) of fructans of vegetables including the scorzonera and the impact of steaming (i.e. cooking 20–30 min using a steam cooker followed by freeze-dried) on these profiles [15]. From those analyses, we can conclude that the DP was higher after steam cooking (13.5 versus 11.7). The proportion of soluble DF was much higher than insoluble DF (more than 70% versus 8%) and this proportion was not affected by the steam. Besides considering DP of fructans and proportion of insoluble proportion of DF, interesting polyphenolic components able to modulate gut microbiota, such as dicaffeoylquinic acids and lignans, were present in *Scorzonera hispanica* commonly known as black salsify (and absent in the purified inulin) [49–51]. It has been already shown that such differences in bioactive substances present in powdered Jerusalem artichokes -rather than inulin supplements- are capable of changing the composition of gut microbiota with beneficial consequences in mice fed a HF diet [52]. In addition, we wondered whether such differences observed on the gut microbiota composition, between Inu and Sco groups, can be partially due to the different amount of cellulose present in the diets (23.7% in HF, 12.7% in Inu versus 5.7% in Sco). We may not rule out that the different cellulose content in the three HF diets induced slight differences in the gut. However, cellulose is an insoluble fibre that is resistant to enzymatic digestion but poorly fermented by the gut microbiota of non-ruminant mammals [53]. Insoluble and poorly fermented fibres, such as cellulose, have a greater effect for reducing the transit time than fermentable fibres (degraded by their fermentation in the colon). In our study, we found a higher feces weight after a 24-h collection in HF-fed mice and the lower weight was found in Sco group. This result is consistent with the ratio of insoluble/soluble fibres since the feces weight decreased in groups with less cellulose incorporated into the diet.

In conclusion, we observed that supplementation with different source of fructans during a HF diet resulted in a shift of the gut microbiota composition, that does not only implicate the increase in *Bifidobacterium* genus, the main target of fructans, but also an increase in gut microbial alpha diversity that was compromised by the HF diet. However, the effects on metabolic alterations differ between the two different sources of ITF. In particular, scorzonera supplementation induced beneficial effects on the intestinal morphology and physiology (crypts depth, antimicrobial peptides, occludin) whereas purified inulin mainly improved hepatic lipids and triglycerides accumulation in a model of HF diet-induced obesity. Our study shows the importance of considering the type of DF source and their presence in food matrix when evaluating their interest in the management of metabolic alterations. The relevance of those observation for human health and nutrition should be addressed, knowing that DF intake is of increasing interest in the control of non-communicable diseases, including obesity [54]. One study investigated the effects of production process of snack bars containing either chicory inulin or Jerusalem artichoke in healthy volunteers during 3 weeks [12]. The authors did not observe structural differences between inulin or Jerusalem artichoke before and after processing and reported similar effects of both inulin and Jerusalem artichoke on the gut microbiota (mainly by enhancing the growth of bifidobacteria) and bowel habit [12]. Such studies are of interest, and would be assorted of comparison of different sources of a similar DF on both biological outcomes and gut microbiota analysis. Finally, this study opens the perspective of the interest of unravelling which gut microbial metabolites could be involved in the improvement of metabolism by inulin-containing food.

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Data availibility All data will be available from the corresponding author on reasonable request.

Declarations

Conflict of interest P.D.C. is inventor on patent applications dealing with the use of specific bacteria and components in the treatment of different diseases. P.D.C. was co-founder of The Akkermansia Company SA and Enterosys. The other authors declare that they have no competing interests.

References

1. Harakeh SM, Khan I, Kumosani T, Barbour E, Almasaudi SB, Bahijri SM, Alfadul SM, Ajabnoor GM, Azhar EI (2016) Gut microbiota: a contributing factor to obesity. Front Cell Infect Microbiol 6:95. https://doi.org/10.3389/fcimb.2016.00095
2. Delzenne NM, Cani PD, Everard A, Neyrinck AM, Bindels LB (2015) Gut microorganisms as promising targets for the management of type 2 diabetes. Diabetologia 58(10):2206–2217. https://doi.org/10.1007/s00125-015-3712-7
3. Van Hul M, Cani PD (2023) The gut microbiota in obesity and weight management: microbes as friends or foe? Nat Rev Endocrinol 19(5):258–271. https://doi.org/10.1038/s41574-022-00794-0
4. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G (2017) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 14(8):491–502. https://doi.org/10.1038/nrgastro.2017.75
5. Cani PD, Lecourt E, Dewulf EM, Sohet FM, Pachikian BD, Naslain D, De Backer F, Neyrinck AM, Delzenne NM (2009) Gut microbiota fermentation of prebiotics increases satietogenic and incretin gut peptide production with consequences for appetite sensation and glucose response after a meal. Am J Clin Nutr 90(5):1236–1243. https://doi.org/10.3945/ajcn.2009.28095
6. Nicolucci AC, Hume MP, Martínez I, Mayengbam S, Walter J, Reimer RA (2017) Prebiotics reduce body fat and alter intestinal microbiota in children who are overweight or with obesity. Gastroenterology 153(3):711–722. https://doi.org/10.1053/j.gastro.2017.05.055
7. Russo F, Linsalata M, Clemente C, Chiloiro M, Orlando A, Marconi E, Chimienti G, Riezzo G (2012) Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. Nutr Res 32(12):940–946. https://doi.org/10.1016/j.nutres.2012.09.010
8. Kalala G, Kambashi B, Taminiau B, Schroyen M, Everaert N, Beckers Y, Richel A, Njeumen P, Pachikian B, Neyrinck AM, Hiel S, Rodriguez J, Fall PA, Daube G, Thissen JP, Delzenne NM, Bindelle J (2020) In vitro approach to evaluate the fermentation pattern of inulin-rich food in obese individuals. Br J Nutr 123(4):472–479. https://doi.org/10.1017/S0007114519002915
9. Neyrinck AM, Nazare JA, Rodriguez J, Jottard R, Dib S, Sothier M, Berghe LVD, Alligier M, Alexiou H, Maquet V, Vinoy S, Bischoff SC, Walter J, Laville M, Delzenne NM (2020) Development of a repertoire and a food frequency questionnaire for estimating dietary fiber intake considering prebiotics: input from the FiberTAG project. Nutrients. https://doi.org/10.3390/nu12092824
10. Hiel S, Bindels LB, Pachikian BD, Kalala G, Broers V, Zamariola G, Chang BPI, Kambashi B, Rodriguez J, Cani PD, Neyrinck AM, Thissen JP, Luminet O, Bindelle J, Delzenne NM (2019) Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. Am J Clin Nutr 109(6):1683–1695. https://doi.org/10.1093/ajcn/nqz001
11. Ramnani P, Gaudier E, Bingham M, van Bruggen P, Tuohy KM, Gibson GR (2010) Prebiotic effect of fruit and vegetable shots containing Jerusalem artichoke inulin: a human intervention study. Br J Nutr 104(2):233–240. https://doi.org/10.1017/S000711451000036X
12. Kleessen B, Schwarz S, Boehm A, Fuhrmann H, Richter A, Henle T, Krueger M (2007) Jerusalem artichoke and chicory inulin in bakery products affect faecal microbiota of healthy volunteers. Br J Nutr 98(3):540–549. https://doi.org/10.1017/S0007114507730751
13. Hiel S, Gianfrancesco MA, Rodriguez J, Portheault D, Leyrolle Q, Bindels LB, da Silveira G, Cauduro C, Mulders M, Zamariola G, Azzi AS, Kalala G, Pachikian BD, Amadieu C, Neyrinck AM, Loumaye A, Cani PD, Lanthier N, Trefois P, Klein O, Luminet O, Bindelle J, Paquot N, Cnop M, Thissen JP, Delzenne NM (2020) Link between gut microbiota and health outcomes in inulin -treated obese patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial. Clin Nutr 39(12):3618–3628. https://doi.org/10.1016/j.clnu.2020.04.005
14. Rodriguez J, Hiel S, Neyrinck AM, Le Roy T, Potgens SA, Leyrolle Q, Pachikian BD, Gianfrancesco MA, Cani PD, Paquot N, Cnop M, Lanthier N, Thissen JP, Bindels LB, Delzenne NM (2020) Discovery of the gut microbial signature driving the efficacy of prebiotic intervention in obese patients. Gut. https://doi.org/10.1136/gutjnl-2019-319726
15. Kalala G, Kambashi B, Everaert N, Beckers Y, Richel A, Pachikian B, Neyrinck AM, Delzenne NM, Bindelle J (2018) Characterization of fructans and dietary fibre profiles in raw and steamed vegetables. Int J Food Sci Nutr 69(6):682–689. https://doi.org/10.1080/09637486.2017.1412404
16. Steegmans M, Iliaens S, Hoebregs H (2004) Enzymatic, spectrophotometric determination of glucose, fructose, sucrose, and inulin/oligofructose in foods. J AOAC Int 87(5):1200–1207
17. Olivares M, Neyrinck AM, Potgens SA, Beaumont M, Salazar N, Cani PD, Bindels LB, Delzenne NM (2018) The DPP-4 inhibitor vildagliptin impacts the gut microbiota and prevents disruption of intestinal homeostasis induced by a Western diet in mice. Diabetologia 61(8):1838–1848. https://doi.org/10.1007/s00125-018-4647-6
18. Escudie F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G (2018) FROGS: Find, Rapidly, OTUs with Galaxy Solution. Bioinformatics 34(8):1287–1294. https://doi.org/10.1093/bioinformatics/btx791
19. Mahe F, Rognes T, Quince C, de Vargas C, Dunthorn M (2015) Swarm v2: highly-scalable and high-resolution amplicon clustering. PeerJ 3:e1420. https://doi.org/10.7717/peerj.1420
20. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R (2011) UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16):2194–2200. https://doi.org/10.1093/bioinformatics/btr381
21. Rognes T, Flouri T, Nichols B, Quince C, Mahe F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584. https://doi.org/10.7717/peerj.2584
22. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO (2013) The SILVA ribosomal RNA gene

database project: improved data processing and web-based tools. Nucleic Acids Res. https://doi.org/10.1093/nar/gks1219
23. Benjamini YHY (1995) Controlling the false discovery rate : a practical and powerful approach to multiple testing. J Roy Stat Soc 57:289–300
24. Cani PD, Dewever C, Delzenne NM (2004) Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br J Nutr 92(3):521–526. https://doi.org/10.1079/bjn20041225
25. Zhong J, Gong Q, Goud A, Srinivasamaharaj S, Rajagopalan S (2015) Recent advances in dipeptidyl-peptidase-4 inhibition therapy: lessons from the bench and clinical trials. J Diabetes Res 2015:606031. https://doi.org/10.1155/2015/606031
26. Olivares M, Schuppel V, Hassan AM, Beaumont M, Neyrinck AM, Bindels LB, Benitez-Paez A, Sanz Y, Haller D, Holzer P, Delzenne NM (2018) The potential role of the dipeptidyl peptidase-4-like activity from the gut microbiota on the host health. Front Microbiol 9:1900. https://doi.org/10.3389/fmicb.2018.01900
27. Hiel S, Neyrinck AM, Rodriguez J, Pachikian BD, Bouzin C, Thissen JP, Cani PD, Bindels LB, Delzenne NM (2018) Inulin improves postprandial hypertriglyceridemia by modulating gene expression in the small intestine. Nutrients. https://doi.org/10.3390/nu10050532
28. Wahlstrom A, Sayin SI, Marschall HU, Backhed F (2016) Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell Metab 24(1):41–50. https://doi.org/10.1016/j.cmet.2016.05.005
29. Ghazi AR, Sucipto K, Rahnavard A, Franzosa EA, McIver LJ, Lloyd-Price J, Schwager E, Weingart G, Moon YS, Morgan XC, Waldron L, Huttenhower C (2022) High-sensitivity pattern discovery in large, paired multiomic datasets. Bioinformatics 38(Suppl 1):i378–i385. https://doi.org/10.1093/bioinformatics/btac232
30. Olivares M, Rodriguez J, Pötgens SA, Neyrinck AM, Cani PD, Bindels LB, Delzenne NM (2019) The janus face of cereals: wheat-derived prebiotics counteract the detrimental effect of gluten on metabolic homeostasis in mice fed a high-fat/high-sucrose diet. Mol Nutr Food Res 63(24):e1900632. https://doi.org/10.1002/mnfr.201900632
31. Neyrinck AM, Van Hée VF, Piront N, De Backer F, Toussaint O, Cani PD, Delzenne NM (2012) Wheat-derived arabinoxylan oligosaccharides with prebiotic effect increase satietogenic gut peptides and reduce metabolic endotoxemia in diet-induced obese mice. Nutr Diabetes 2(1):e28. https://doi.org/10.1038/nutd.2011.24
32. Rodriguez J, Delzenne NM (2021) Modulation of the gut microbiota-adipose tissue-muscle interactions by prebiotics. J Endocrinol 249(1):R1–R23. https://doi.org/10.1530/JOE-20-0499
33. Alvarez-Sola G, Uriarte I, Latasa MU, Fernandez-Barrena MG, Urtasun R, Elizalde M, Barcena-Varela M, Jiménez M, Chang HC, Barbero R, Catalán V, Rodríguez A, Frühbeck G, Gallego-Escuredo JM, Gavaldà-Navarro A, Villarroya F, Rodriguez-Ortigosa CM, Corrales FJ, Prieto J, Berraondo P, Berasain C, Avila MA (2017) Fibroblast growth factor 15/19 (FGF15/19) protects from diet-induced hepatic steatosis: development of an FGF19-based chimeric molecule to promote fatty liver regeneration. Gut 66(10):1818–1828. https://doi.org/10.1136/gutjnl-2016-312975
34. Liu XJ, Duan NN, Liu C, Niu C, Liu XP, Wu J (2018) Characterization of a murine nonalcoholic steatohepatitis model induced by high fat high calorie diet plus fructose and glucose in drinking water. Lab Invest 98(9):1184–1199. https://doi.org/10.1038/s41374-018-0074-z
35. Paone P, Suriano F, Jian C, Korpela K, Delzenne NM, Van Hul M, Salonen A, Cani PD (2022) Prebiotic oligofructose protects against high-fat diet-induced obesity by changing the gut microbiota, intestinal mucus production, glycosylation and secretion. Gut Microbes 14(1):2152307. https://doi.org/10.1080/19490976.2022.2152307
36. Niu B, Li F, Lv X, Xiao Y, Zhu J, Zhao J, Lu W, Chen W (2024) Unveiling the therapeutic potential and mechanism of inulin in DSS-induced colitis mice. Int J Biol Macromol 280(Pt 2):135861. https://doi.org/10.1016/j.ijbiomac.2024.135861
37. Xie J, Yu R, Qi J, Zhang G, Peng X, Luo J (2020) Pectin and inulin stimulated the mucus formation at a similar level: An omics-based comparative analysis. J Food Sci 85(6):1939–1947. https://doi.org/10.1111/1750-3841.15163
38. Everard A, Lazarevic V, Gaïa N, Johansson M, Ståhlman M, Backhed F, Delzenne NM, Schrenzel J, François P, Cani PD (2014) Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity. Isme j 8(10):2116–2130. https://doi.org/10.1038/ismej.2014.45
39. Beisner J, Filipe Rosa L, Kaden-Volynets V, Stolzer I, Günther C, Bischoff SC (2021) Prebiotic inulin and sodium butyrate attenuate obesity-induced intestinal barrier dysfunction by induction of antimicrobial peptides. Front Immunol 12:678360. https://doi.org/10.3389/fimmu.2021.678360
40. Neyrinck AM, Taminiau B, Walgrave H, Daube G, Cani PD, Bindels LB, Delzenne NM (2017) Spirulina protects against hepatic inflammation in aging: an effect related to the modulation of the gut microbiota? Nutrients. https://doi.org/10.3390/nu9060633
41. Delzenne NM, Bindels LB, Neyrinck AM, Walter J (2024) The gut microbiome and dietary fibres: implications in obesity, cardiometabolic diseases and cancer. Nat Rev Microbiol. https://doi.org/10.1038/s41579-024-01108-z
42. Salazar N, Dewulf EM, Neyrinck AM, Bindels LB, Cani PD, Mahillon J, de Vos WM, Thissen JP, Gueimonde M, de Los Reyes-Gavilán CG, Delzenne NM (2015) Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. Clin Nutr 34(3):501–507. https://doi.org/10.1016/j.clnu.2014.06.001
43. Verbeke KA, Boobis AR, Chiodini A, Edwards CA, Franck A, Kleerebezem M, Nauta A, Raes J, van Tol EA, Tuohy KM (2015) Towards microbial fermentation metabolites as markers for health benefits of prebiotics. Nutr Res Rev 28(1):42–66. https://doi.org/10.1017/s0954422415000037
44. Singh SB, Carroll-Portillo A, Lin HC (2023) Desulfovibrio in the gut: the enemy within? Microorganisms. https://doi.org/10.3390/microorganisms11071772
45. Plovier H, Everard A, Druart C, Depommier C, Van Hul M, Geurts L, Chilloux J, Ottman N, Duparc T, Lichtenstein L, Myridakis A, Delzenne NM, Klievink J, Bhattacharjee A, van der Ark KC, Aalvink S, Martinez LO, Dumas ME, Maiter D, Loumaye A, Hermans MP, Thissen JP, Belzer C, de Vos WM, Cani PD (2017) A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. Nat Med 23(1):107–113. https://doi.org/10.1038/nm.4236
46. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD (2013) Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 110(22):9066–9071. https://doi.org/10.1073/pnas.1219451110
47. Cani PD, Depommier C, Derrien M, Everard A, de Vos WM (2022) Akkermansia muciniphila: paradigm for next-generation beneficial microorganisms. Nat Rev Gastroenterol Hepatol 19(10):625–637. https://doi.org/10.1038/s41575-022-00631-9
48. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis

JD (2011) Linking long-term dietary patterns with gut microbial enterotypes. Science 334(6052):105–108. https://doi.org/10.1126/science.1208344
49. Chu Z, Hu Z, Luo Y, Zhou Y, Yang F, Luo F (2023) Targeting gut-liver axis by dietary lignans ameliorate obesity: evidences and mechanisms. Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2023.2272269
50. Granica S, Lohwasser U, Jöhrer K, Zidorn C (2015) Qualitative and quantitative analyses of secondary metabolites in aerial and subaerial of Scorzonera hispanica L. (black salsify). Food Chem 173:321–331. https://doi.org/10.1016/j.foodchem.2014.10.006
51. Xie M, Chen G, Wan P, Dai Z, Hu B, Chen L, Ou S, Zeng X, Sun Y (2017) Modulating effects of dicaffeoylquinic acids from ilex kudingcha on intestinal microecology in vitro. J Agric Food Chem 65(47):10185–10196. https://doi.org/10.1021/acs.jafc.7b03992
52. Sasaki H, Lyu Y, Nakayama Y, Nakamura F, Watanabe A, Miyakawa H, Nakao Y, Shibata S (2020) Combinatorial effects of soluble, insoluble, and organic extracts from jerusalem artichokes on gut microbiota in mice. Microorganisms. https://doi.org/10.3390/microorganisms8060954
53. Gill SK, Rossi M, Bajka B, Whelan K (2021) Dietary fibre in gastrointestinal health and disease. Nat Rev Gastroenterol Hepatol 18(2):101–116. https://doi.org/10.1038/s41575-020-00375-4
54. Huwiler VV, Schönenberger KA, Segesser von Brunegg A, Reber E, Mühlebach S, Stanga Z, Balmer ML (2022) Prolonged isolated soluble dietary fibre supplementation in overweight and obese patients: a systematic review with meta-analysis of randomised controlled trials. Nutrients. https://doi.org/10.3390/nu14132627