Fermentable Carbohydrate Alters Hypothalamic Neuronal Activity and Protects Against the Obesogenic Environment

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Obesity has become a major global health problem. Recently, attention has focused on the benefits of fermentable carbohydrates on modulating metabolism. Here, we take a system approach to investigate the physiological effects of supplementation with oligofructose-enriched inulin (In). We hypothesize that supplementation with this fermentable carbohydrate will not only lead to changes in body weight and composition, but also to modulation in neuronal activation in the hypothalamus. Male C57BL/6 mice were maintained on a normal chow diet (control) or a high fat (HF) diet supplemented with either oligofructose-enriched In or corn starch (Cs) for 9 weeks. Compared to HF+Cs diet, In supplementation led to significant reduction in average daily weight gain (mean ± s.e.m.: 0.19 ± 0.01 g vs. 0.26 ± 0.02 g, $P < 0.01$), total body adiposity (24.9 ± 1.2% vs. 30.7 ± 1.4%, $P < 0.01$), and lowered liver fat content (11.7 ± 1.7% vs. 23.8 ± 3.4%, $P < 0.01$). Significant changes were also observed in fecal bacterial distribution, with increases in both *Bifidobacteria* and *Lactobacillius* and a significant increase in short chain fatty acids (SCFA). Using manganese-enhanced MRI (MEMRI), we observed a significant increase in neuronal activation within the arcuate nucleus (ARC) of animals that received In supplementation compared to those fed HF+Cs diet. In conclusion, we have demonstrated for the first time, in the same animal, a wide range of beneficial metabolic effects following supplementation of a HF diet with oligofructose-enriched In, as well as significant changes in hypothalamic neuronal activity.

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INTRODUCTION

Obesity has become a major worldwide epidemic with incidence rates above 20% in most Western countries and is a major underlying cause of type 2 diabetes mellitus, coronary heart disease, and susceptibility to a variety of cancers (1). The rise in obesity is fuelled by the mismatch between an inherited genetic background predisposed to survival in environments where food supply is limited and the reduced physical activity and excess energy intake of modern society (2). Modern food processing has resulted in the mass production of cheap energy dense foods that are generally high in refined sugars and fats but low in fibre (2). It has been estimated that the Paleolithic diet delivered more than 100 g/day of fibre whereas current Western intakes are between 10 and 20 g/day (3). Much of the fibre consumed in the Paleolithic diet would have been fermented by the colonic bacteria (3) and there is a growing body of evidence that links fermentation of dietary carbohydrate such as prebiotic inulin (In)-type fructans and resistant starch to positive effects on metabolism (4).

In and oligofructose are natural food ingredients found in plants such as onion, garlic, and chicory. In and oligofructose are indigestible by human small intestinal enzymes but fully fermented by the colonic microbiota (5). An important property of In-type fructans is their action as a prebiotic; they are known to change the intestinal bacteria distribution, allowing beneficial strains such as *Bifidobacteria* to thrive while decreasing levels of harmful pathogenic bacteria (5). A placebo-controlled human study, where 10 g of In was given per day for 3 weeks, with a high-carbohydrate, low-fat diet was found to decrease hepatic lipogenesis and plasma triacylglycerol concentrations (6). Animal studies mirror these findings, with resistant starch supplementation linked to decreases in visceral adipose tissue and hepatic lipid storage, production of small insulin sensitive adipocytes and an overall increase in insulin

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sensitivity (7). Recent observations by Cani *et al.* demonstrated that rats exposed to an increased oligofructose feed, followed by 15 days of high fat (HF) diet had lower energy intake, body weight, body fat, and triglyceride levels suggesting that exposure to a fermentable fibre source leads to improved appetite regulation (8). A consistent finding of these studies is that animals fed fermentable carbohydrate have higher circulating levels of appetite inhibiting gut hormones, such as glucagon-like peptide 1 (GLP-1) and peptide YY and lower levels of the appetite stimulant ghrelin (7,8).

Fermentable carbohydrates such as In and oligofructose are fermented by the colonic bacteria to short chain fatty acids (SCFA) (9). The main SCFA are acetate, propionate, and butyrate and they exist in a ratio of 60:20:20 (9). The SCFA are raised in peripheral circulation following feeding of In and oligofructose (10). There is evidence to suggest that the SCFA, acetate, can travel across the blood–brain barrier into the central nervous system (11). It has been known for some time from work on alcohol metabolism and electrophysiological studies that acetate has central neurological effects (12). This raises the possibility that SCFA may have a direct effect on central neuronal processing and appetite regulation.

Manganese-enhanced magnetic resonance imaging (MEMRI) is a functional imaging technique that has been successfully employed to study neuronal pathways of appetite regulation throughout the central nervous system, *in vivo* (13). The technique relies upon the ability of manganese (Mn^{2+}) ions to mimic calcium ions, thereby entering cells via voltage-gated calcium channels following neuronal activation. Mn^{2+} ions are also paramagnetic, altering signal intensity (SI) in specifically weighted MRI scans and these properties have facilitated its use as an indirect marker of neuronal activation (14). Our laboratory has previously shown that Mn^{2+} ions are capable of entering key appetite centers within the central nervous system, including the hypothalamus and brainstem, enabling the detection of significant differences in activity-dependent Mn^{2+} uptake in response to a diverse range of stimuli, including fed and fasted states and a variety of gut peptides (15,16). Furthermore, the reproducibility observed in the patterns of hypothalamic MEMRI SI following these stimuli contrasts sharply with conflicting data from studies examining protein markers of neuronal activation (such as c-fos) (13).

Here we have used a HF energy-dense diet as a model of the obesogenic environment and supplemented it with oligofructose-enriched In to further investigate the physiological effects of this fermentable carbohydrate. We hypothesize that hypercaloric feeds enriched with fermentable fibre would cause reduced weight gain and an improvement in body composition and metabolic function in mice.

METHODS AND PROCEDURES

Materials

All diets were manufactured by Harlan Teklad (Madison, WI) with oligofructose-enriched inulin (ORAFTISynergy 1) supplied by BENEO-Orafti (Tienen, Belgium). All other chemicals were purchased from Sigma-Aldrich (Poole, UK) unless otherwise stated.

Animals

All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. Male C57Bl/6 mice (8 weeks old; Harlan, Hillcrest, UK) were randomized into dietary treatment groups by weight (data not shown). Animals were housed in individual cages under conditions of controlled temperature (21–23 °C) and light (12 h light, 12 h dark cycle; lights on at 07:00 h) and allowed *ad libitum* access to drinking water and their specific diet.

Experiment 1: the effects of HF diet supplementation with oligofructose-enriched In and Cs on body weight, energy intake, plasma GLP-1, and microbiota

Male C57BL/6 mice were maintained on a HF (21%) diet supplemented with either 7.5% oligofructose-enriched In (HF+In, $n = 15$) or 7.5% Cs (HF+Cs, $n = 13$), with energy contents of 18.0 and 19.2 kJ/g (4.3 and 4.6 kcal/g), respectively, similar to that used by other groups (8). A third group (control) was given a normal chow diet (4% fat) with an energy content of 14.6 kJ/g (3.5 kcal/g, control, $n = 8$). The energy density of the HF+In diet was slightly lower than that of the HF+Cs diet. Dietary constituents expressed as percentages by weight, are shown in Table 1. Animals were fed *ad libitum* for a period of 9 weeks, with body weight and food intake recorded daily. Frozen fecal and cecal samples were taken at the start of the study ($t = 0$ weeks) and following 8 weeks of dietary supplementation ($t = 8$ weeks). Samples were subjected to fluorescent *in situ* hybridization with Cy-3 labeled genus specific probes, Lab158, Bif 164, Erec 482, and Mib 663 (17,18) to enumerate *Lactobacillus-Enterococcus*, *Bifidobacteria*, *Eubacterium rectale-Clostridium coccoides*, and mouse intestinal bacteria, respectively, as previously described. Total cell counts were obtained with DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) stain (19). After 9 weeks of dietary manipulation serum GLP-1 concentrations were measured using an in-house radioimmunoassay as previously described (20).

SCFA in the colonic content were determined by gas chromatography analysis. The method used was adapted from Richardson *et al.* (21). whereby organic acids were extracted and t-BDMS (tert-butyl dimethylsilyl) derivatives formed. Cecal contents were weighed (20–220 µg) and combined with 550 µl of phosphate-buffered saline. Samples were vortex mixed for 1 min, centrifuged at 3,000*g* for 10 min and the supernatant collected. To 500 µl supernatant, 25 µl of internal standard, 2-ethylbutyric acid, was added to give a final concentration of 5 mmol/l. Acids were extracted by the addition of 250 µl concentrated hydrochloric acid and 1 ml diethyl ether followed by vortex mixing for 1 min. Samples were centrifuged for 10 min at 3,000*g* and the ether layer removed and transferred to a separate capped vial. *N*-methyl-*N*-t-butyldimethylsilyltrifluoroacetamide (MTBSTFA; Sigma-Aldrich)

Table 1 Nutritional composition of diets showing the amount of metabolizable protein, carbohydrate, and fat % by weight and the energy in kJ/g

	HF+Cs	HF+In	Control
Protein % by weight	17.3	17.3	20.2
Carbohydrate % by weight	49.0	42.3[a]	33.9
Fat % by weight	21.2	21.2	3.9
Fibre % by weight	5	12.5[b]	15.4
Energy kJ/g	19.2	19.0[a]	11.4
Inulin % by weight	0	7.5	0

HF+Cs and HF+In contain 21% anhydrous milk-fat and 34% sucrose. HF+Cs contain 7.5% corn starch instead of the oligofructose-enriched inulin in the HF+In diet.
Cs, corn starch; HF, high fat; In, inulin.
[a]Energy for the HF+In includes an estimate of energy gained from fermentation.
[b]In the HF+In diet, 7.5% of the fibre content is oligofructose-enriched inulin.

was added (100 µl) before heating at 80 °C for 20 min. Gas chromatography was performed on a Hewlett Packard 5890 Series II instrument equipped with a flame ionization detector, split/splitless injector and a 10 m, 0.18 mm ID and 0.20 µm df Rtx-1 (Crossbond 100% dimethyl polysiloxane; Thames Restek, Saunderton, UK) capillary column. Injector and detector temperatures were 275 °C with the column temperature programmed from 63 °C for 3 min to 190 °C at 10 °C/min. Helium served as the carrier gas (head pressure 135 kPa) and injections (1 µl) were made in the split mode (50:1 split). Peak areas were recorded and all subsequent data manipulation was completed using ChemStation Software (Agilent Technologies, Santa Clara, CA). External standards for acetate, propionate, *n*-butyrate, iso-butyrate, *n*-valerate, and caproate were prepared at concentrations of 25, 12.5, 6.25, 1.25, and 0.625 mmol/l and ethyl butyric acid was used as the internal standard at a concentration of 100 mmol/l. Reported values were normalized according to the weight of original sample used.

Experiment 2: the effects of HF diet supplementation with oligofructose-enriched In on whole body adiposity and intrahepatocellular lipid

Following 8 weeks of dietary supplementation (as above) whole body adiposity was determined by MRI. We have used MRI to differentiate between subcutaneous and internal adipose tissue and to derive quantitative data for volumes of each of these reserves (22). Mice were anesthetized with 3% isoflurane in oxygen at a flow-rate of 2 l/min. Scans were acquired on a 4.7 Tesla Varian INOVA imaging system (Varian, Palo Alto, CA) using a quadrature birdcage coil. Contiguous axial MR images of the whole mouse body were collected using a spin-echo sequence with parameters: repetition time (TR) 2.2 s, echo time (TE) 20 ms, field of view 45 mm × 45 mm, matrix 256 × 192, 2 averages, and 2 mm thick slices. Segmentation analysis was performed with SliceOmatic (Tomovision, Montreal, Quebec, Canada) to provide volumes (and masses) of internal and subcutaneous adipose tissue deposits, as previously described (23). In addition, we used localized ^{1}H MRS to measure relative amounts of intrahepatocellular lipid (IHCL) following 8 weeks of dietary supplementation. Spectroscopy was performed using a point-resolved spectroscopy (PRESS) sequence with TR 10 s, TE 9 ms, and 64 averages following voxel (2 × 2 × 2 mm) placement by MRI. The spectra were analyzed using MestRe-C (MestReLab Research, Santiago de Compostela, Spain) where an exponential line broadening of 1.5 Hz was applied, before baseline correction and integration of the lipid peak with normalization to the water peak which was arbitrarily assigned as being 100%, as previously described (7).

Experiment 3: the effects of HF diet supplementation with oligofructose-enriched In on hypothalamic activity as measured by MEMRI

After 9 weeks of dietary manipulation MEMRI scans were performed, on *ad libitum* fed mice that received a HF diet with (HF+In, $n = 7$) or without (HF+Cs, $n = 8$) In supplementation, as previously described (24). Briefly, MEMRI analysis of the brain was performed on a 9.4 Tesla Varian INOVA imaging system (Varian) using a quadrature birdcage coil. Forty-six transverse contiguous slices (0.4 mm thick) were acquired repeatedly (66 times over a period of ~2 h) using a fast spin-echo sequence with parameters: TR 1.8 s, TEeff 5.6 ms, field of view 25 mm × 25 mm, matrix 192 × 192, and 2 averages. After the third acquisition (3/66), 100 mmol/l $MnCl_2$ was infused intravenously (2 ml/h, 0.5 µmol/g body weight). Image J (NIH, Bethesda, MD) was used to measure SI changes from the resulting scan images in specific regions of interest (ROI); the arcuate nucleus (ARC), ventromedial hypothalamus, paraventricular nucleus, and periventricular nucleus (**Figure 1**). The ROIs were placed on each image with careful reference to a standard brain atlas (25). The specific regions were not covered in their entirety, due to the differences in slice thickness acquired by our protocol and those recorded in the mouse brain atlas. However, the majority of the desired region was within the ROI, while enough was left on all sides in order to avoid overlap with other regions. The percentage change in SI from baseline (acquisition before Mn^{2+} infusion) in each ROI were normalized to the anterior pituitary gland to account for differences in manganese uptake across the blood–brain barrier.

Statistics

Data are presented as means ± s.e.m. Comparisons of percentage adiposity (mass of adipose tissue by MRI segmentation analysis normalized to body weight), and IHCL content between the HF+In, HF+Cs, and control groups were performed using ANOVA with Bonferroni post-testing. Differences in SI profile, body weight, and cumulative food intake in all experimental groups were analyzed using

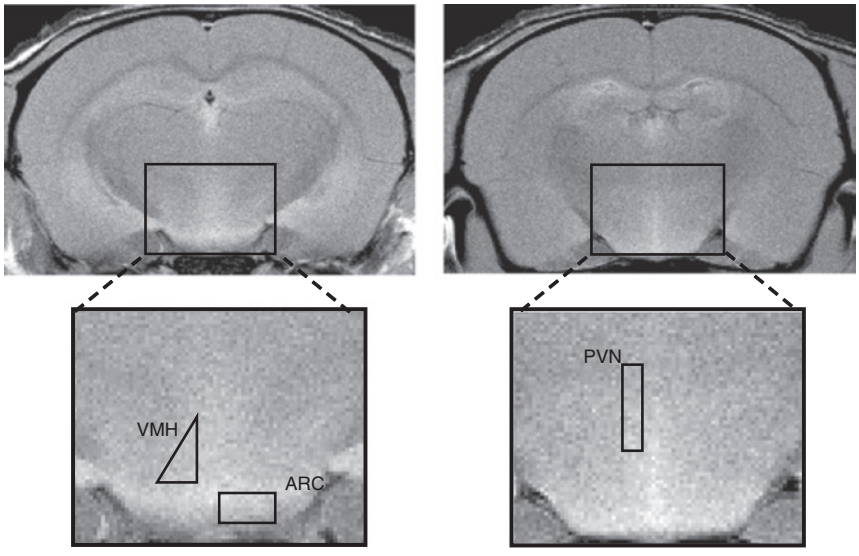

Figure 1 Regions of interest (ROIs) used in MEMRI analysis. MR image showing the ROI locations in the hypothalamus from which SI measurements were determined. ARC, arcuate nucleus; MEMRI, manganese-enhanced magnetic resonance imaging; MR, magnetic resonance; PVN, paraventricular nucleus; SI, signal intensity; VMH, ventromedial hypothalamus.

generalized estimating equation and the Mann–Whitney U-test in commercial statistical software (Stata 9.1; Stata, College Station, TX). MEMRI data was analyzed between dietary groups by the SI profiles using the generalized estimating equation as previously described (15,16). Acetate and propionate feeding study data was analyzed using a two-tailed *t*-test.

RESULTS

Experiment 1: the effects of HF diet supplementation with oligofructose-enriched In and Cs on body weight, energy intake, plasma GLP-1 and microbiota

The supplementation of a HF diet with oligofructose-enriched inulin (HF+In) resulted in a reduction in body weight compared to corn starch fed controls (HF+Cs), becoming significant 15 days following dietary manipulation (day 15 body weight: HF+In: 27.3 ± 0.6 g vs. HF+Cs: 28.9 ± 0.7 g; $P < 0.05$) (**Figure 2a**). This difference was maintained until the end of the study. Oligofructose-enriched In supplementation resulted in a body weight not significantly different to that of normal chow fed controls (**Figure 2a**). Significant differences were recorded between the average daily body weight change between all three groups (**Figure 2b**) (HF+Cs: 0.26 ± 0.02 g, HF+In: 0.19 ± 0.01 g, control: 0.09 ± 0.02 g; HF+Cs vs. HF+In: $P < 0.01$, HF+In vs. control: $P < 0.001$). We also recorded a significant increase in cumulative energy intake in both HF dietary groups compared to the control group, manifesting at day 10 and lasting for the remainder of the study (**Figure 2c**). A significant increase in cumulative energy feeding was observed in the HF+Cs compared to the HF+In group at day 19, and lasted for the remainder of the study (**Figure 2c**). There was a decreased total caloric intake in the HF+In mice compared to those fed the HF+Cs diet ($P < 0.01$, **Figure 2d**).

Oligofructose-enriched In supplementation resulted in an increase in *Bifidobacteria* in the cecal contents compared to HF+Cs mice (cecal *Bifidobacteria*; HF+In: 8.5 ± 0.1 vs. HF+Cs: 7.1 ± 0.1, $P < 0.001$, **Figure 3a**). In addition, dietary supplementation with oligofructose-enriched In resulted in a change in fecal bacteria distribution following 8 weeks of feeding (fecal *Bifidobacteria* at $t = 0$ weeks: HF+In: 6.5 ± 0.1 vs. HF+Cs: 6.6 ± 0.2, $P = 0.53$; $t = 8$ weeks; HF+In: 8.8 ± 0.1 vs. HF+Cs: 7.3 ± 0.2, $P < 0.001$, **Figure 3b**). A similar effect was also observed regarding the fecal *Lactobacillus-Enterococcus* concentration after 8 weeks feeding period (fecal *Lactobacillus-Enterococcus* at $t = 0$ weeks; $P = 0.13$, $t = 8$ weeks; $P < 0.01$, **Figure 3b**). There was a trend towards increased GLP-1 serum concentration in the HF+In group compared to HF+Cs mice but this did not reach significance (GLP-1 serum levels: HF+In: 57.8 ± 13.0 pmol/l vs. control: 35.2 ± 6.2 pmol/l; $P = 0.19$).

Total colonic content of SCFA (acetate+propionate+butyrate) was significantly higher in the HF+In group compared to the HF+Cs group (HF+In: 34.4 ± 2.5 mmol/l; HF+Cs: 17.7 ± 4.1 mmol/l, $P < 0.05$). Acetate and propionate levels were significantly increased in HF+In compared to HF+Cs (acetate 25.6 ± 2.8 mmol/l vs. 12.6 ± 4.4 mmol/l: propionate 4.3 ± 1.1 mmol/l vs. 1.2 ± 0.6 mmol/l respectively, $P < 0.05$). There was no difference in the butyrate content

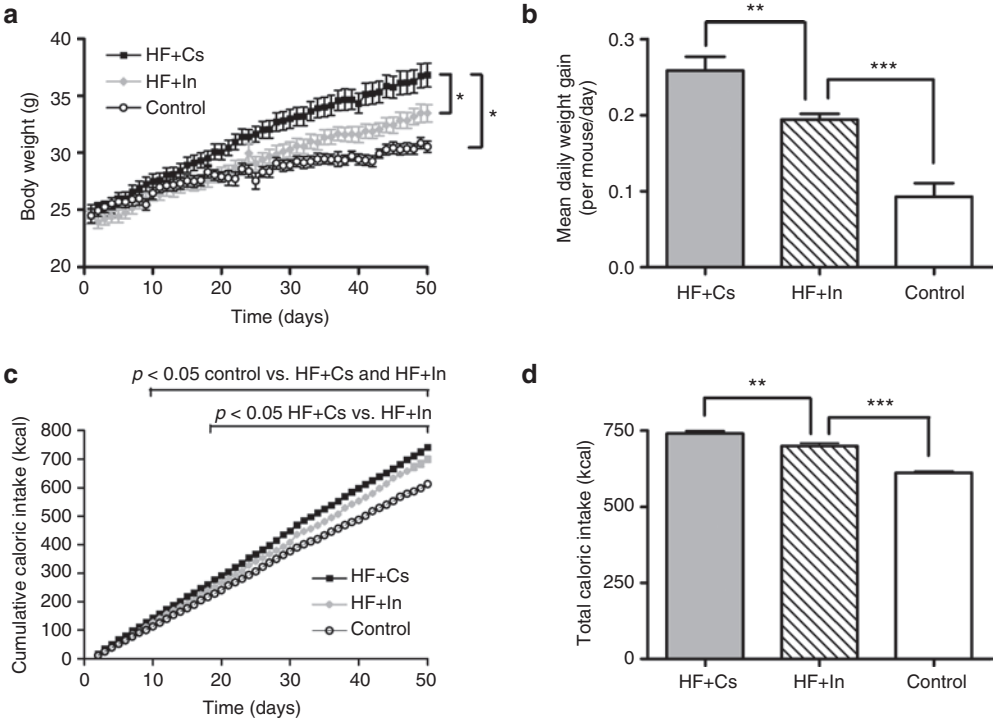

Figure 2 The effects of oligofructose-enriched inulin supplementation on body weight and daily caloric intake. Mice were fed normal chow (control, $n = 8$) diet or a high-fat diet containing either oligofructose-enriched inulin (HF+In, $n = 15$), or nonfermentable corn starch (HF+Cs, $n = 13$) *ad libitum* for 8 weeks. The effects on (**a**) body weight, (**b**) mean daily weight gain, (**c**) cumulative caloric intake, and (**d**) total caloric intake are shown; ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Cs, corn starch; HF, high fat, In, inulin.

Experiment 2: the effects of HF diet supplementation with oligofructose-enriched In on whole-body adiposity and IHCL

HF+In mice demonstrated a lower percentage whole-body adiposity compared to HF+Cs mice (whole-body percentage adiposity: HF+In: 24.9 ± 1.2% vs. HF+Cs: 30.7 ± 1.4%, $P < 0.01$, **Figure 4a**). There was a significant decrease in both subcutaneous and internal adipose tissue in the HF+In compared to the HF+Cs group (subcutaneous fat: HF+In: 4.5 ± 0.3 g vs. HF+Cs: 6.0 ± 0.4 g; $P < 0.01$; internal fat: HF+In: 3.4 ± 0.3 g vs. HF+Cs: 4.9 ± 0.4 g; $P < 0.01$) (**Supplementary Figures S1** and **S2** online). The ratio of subcutaneous and internal adipose tissue was comparable between the HF+In and HF+Cs groups (HF+In; 1.3 ± 0.1 vs. HF+Cs; 1.3 ± 0.1, arbitrary units) (data not shown). Mice fed the HF+In diet showed significantly lower IHCL than those fed the HF+Cs diet (IHCL: HF+In: 11.7 ± 1.7% vs. HF+Cs: 23.8 ± 3.4%; $P < 0.01$) (**Figure 4b**). Furthermore, IHCL levels in the HF+In group were not significantly different from that of control animals fed a reduced-fat diet (IHCL: HF+In: 11.7 ± 1.7% vs. control: 9.0 ± 1.0%; $P = 0.29$).

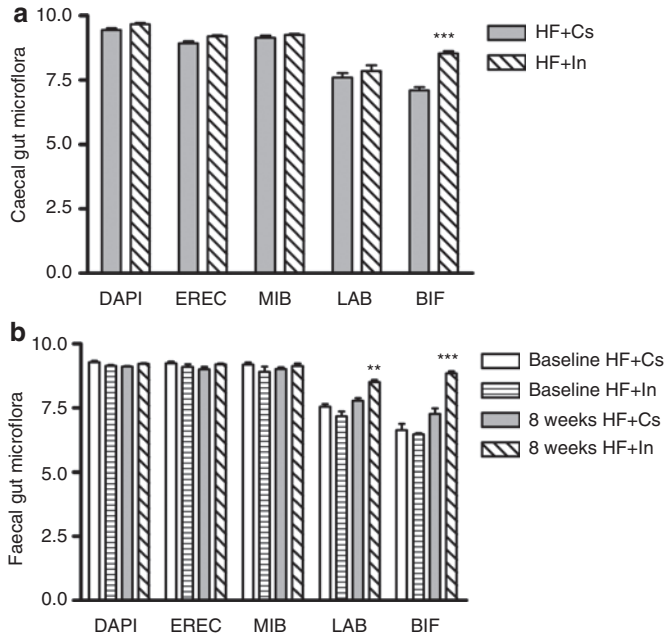

Figure 3 The effects on microbiota of dietary oligofructose-enriched inulin supplementation. The microbiota in the (**a**) caecum and (**b**) the faeces of *ad libitum* fed mice given either oligofructose-enriched inulin (HF+In, $n = 8$) or corn starch (HF+Cs, $n = 8$) dietary supplementation. BIF, *Bifidobacteria*; Cs, corn starch; DAPI, 4′,6-diamidino-2-phenylindole dihydrochloride; EREC, *Eubacterium rectale–Clostridium coccoides*; HF, high fat; In, inulin; LAB, *Lactobacillus-Enterococcus*; MIB, mouse intestinal bacteria. ***: $P < 0.001$, **P: < 0.01.

Experiment 3: the effects of HF diet supplementation with oligofructose-enriched In on hypothalamic activity as measured by MEMRI

MEMRI analysis revealed a significant increase in SI in the ARC of HF+In animals compared to the HF+Cs group (ARC SI: HF+Cs vs. HF+In, $P < 0.05$) (**Figure 5a**). No additional, significant differences were observed between the SI profiles of HF+In and HF+Cs groups in the ventromedial hypothalamus ($P = 0.86$, **Figure 5b**), paraventricular nucleus ($P = 0.66$, **Figure 5c**), or the brainstem ($P = 0.20$, **Figure 5d**).

DISCUSSION

Here, we demonstrate for the first time in the single animal model that the fermentable carbohydrate In causes significant change in body weight, energy intake, fat deposition, microbiota, SCFA concentration, and hypothalamic activity when used to supplement a HF, energy-dense feed. Reduced weight gain following In supplementation has previously been recorded in both dietary obese animal models (26) and also extends to humans; with a recent feeding study demonstrating lower weight gain in those who received a dietary supplement of In and a decrease in appetite (27). The small but sustained decrease in daily energy intake we have recorded in the HF+In group is likely responsible for the reductions in total body-weight gain, whole-body adiposity, and ectopic lipid distribution seen when compared to high-fat controls. These changes are in agreement with previous publications; a significant reduction in whole-body fat mass was observed in rodents fed long-chain In for 4 and 8 weeks (28), while Cani *et al.* showed similar effects on body composition in rats fed a high oligofructose diet for 35 days and then exposed to a HF diet (8). We have previously demonstrated a reduction in

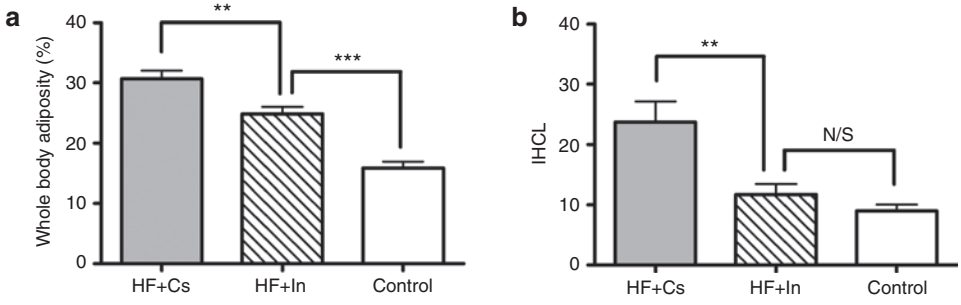

Figure 4 The effects of oligofructose-enriched inulin supplementation on whole body adiposity and intrahepatocellular lipid (IHCL). (**a**) Whole body adiposity and (**b**) IHCL content were determined in mice fed normal chow (control, $n = 4$) or a high-fat diet supplemented with either oligofructose-enriched inulin (HF+In, $n = 7$) or nonfermentable corn starch (HF+Cs, $n = 8$). Data analyzed using ANOVA with Bonferonni post-testing; ***$P < 0.001$, **$P < 0.01$, P = NS. Cs, corn starch; HF, high fat; In, inulin; NS, nonsignificant.

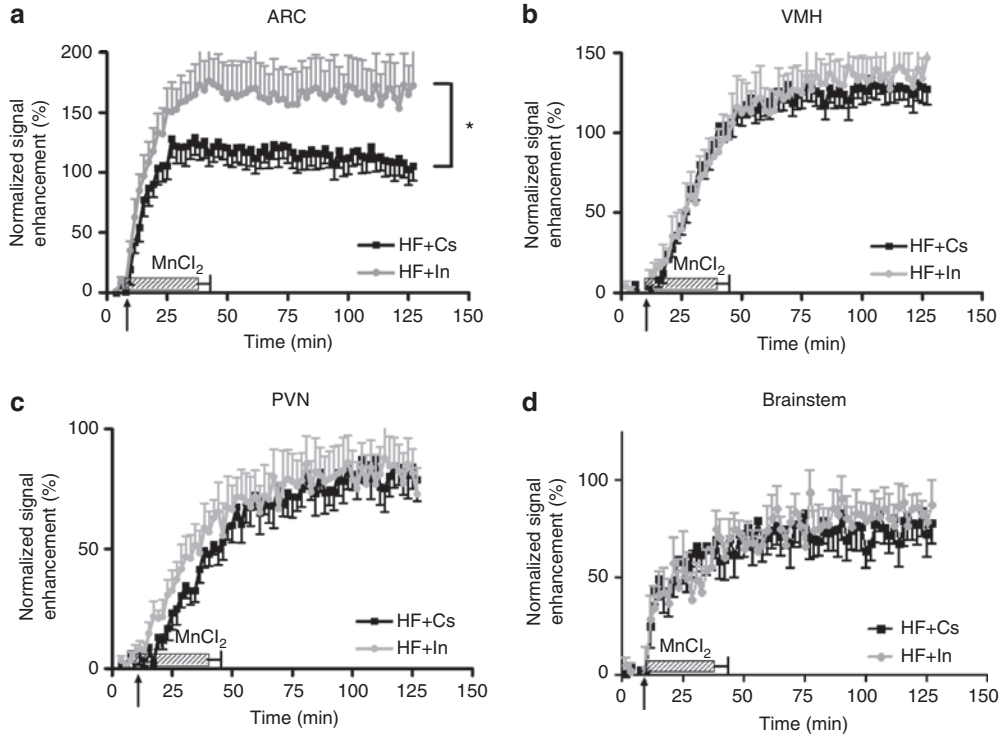

Figure 5 The effects of dietary oligofructose-enriched inulin supplementation on hypothalamic signaling in *ad libitum* fed mice. Time course of normalized T1-weighted MEMRI SI change (normalized percentage enhancement) after intravenous $MnCl_2$ infusion recorded in the (**a**) ARC, (**b**) VMH, and (**c**) PVN, in *ad libitum* fed mice with dietary supplementation with oligofructose-enriched inulin (HF+In, $n = 7$) or corn starch (HF+Cs, $n = 8$). The arrow indicates the start of intravenous $MnCl_2$ infusion, the hatched bar indicates the duration of the intravenous infusion. Statistical analysis was carried out using GEE; $^*P < 0.05$. ARC, arcuate nucleus; Cs, corn starch; GEE, generalized estimating equation; HF, high fat; In, inulin; MEMRI, manganese-enhanced magnetic resonance imaging; PVN, paraventricular nucleus; SI, signal intensity; VMH, ventromedial hypothalamus.

internal compared to subcutaneous adipose tissue deposits in animals fed high levels of resistant starch on a normal fat diet (7). While we have not observed a similar difference in the subcutaneous to internal adipose tissue ratio following In supplementation, the reduction in internal adipose tissue is likely to be metabolically advantageous.

A further metabolic advantage of oligofructose-enriched In supplementation may be the significant reduction in hepatic lipid content we have recorded using ^{1}H MRS. Similar effects of oligofructose-enriched In supplementation have been recorded in rats (26), and indirectly in humans; showing both a reduction in hepatic lipogenesis (8) and an improvement in hepatic enzymes in subjects with non-alcoholic steatohepatitis (29). It is possible that In affects energy expenditure via upregulation of lipid oxidation; dietary supplementation with In in both rats (30) and humans (6) has been shown to reduce serum triacylglycerol levels. This mechanism may involve SCFA, such as propionate, produced from the fermentation of carbohydrate, stimulating peroxisome proliferator-activated receptor-α, and inducing lipid oxidation.

In agreement with previous data, we show that oligofructose-enriched In supplementation alters cecal and fecal distribution of microflora by increasing the population of the beneficial *Bifidobacteria* and *Lactobacillus-Enterococcus* species. Previous research studying obese *fa/fa* rat models has shown a correlation between a reduction in weight and a decrease in the number of *Bifidobacteria* with occurrence of obesity (31). Furthermore, oligofructose feeding has been shown to reduce the body-weight gain and fat mass development in HF fed *Ob/Ob* mice, which could be ascribed to an increase in *Bifidobacteria* numbers and a fall in intestinal endotoxin levels (32). It is of interest that the effects of fermentable fibres on body composition are lost if fermentation is prevented (33).

The shift in the microbiota population levels in our study, following In supplementation, towards an increase in *Bifidobacteria* correlates well with the increase in circulating GLP-1 which we recorded in these mice. Previous studies have shown that dietary treatment with oligofructose in rats, which has been shown to alter the gut microbiota population, can increase the levels of GLP-1 as well as alter the number of GLP-1 producing colonic cells, L-cells (34). A different study also showed that the intestinal microbiota can influence the gastrointestinal epithelium structure, including numbers of endocrine crypt cells (35). In our experiment, we have demonstrated an increase in *Bifidobacteria* and *Lactobacillus-Enterococcus* species, as well as in colonic content of acetate and pyruvate, in mice given In, with a HF diet. SCFA are thought to contribute to an increased production of GLP-1 and other gut hormones by the colonic endocrine cells (36). The increase in GLP-1 which we observed following In supplementation could be a direct consequence of

the increase in SCFA, which are produced by microbiota fermentation. We have shown that In causes this combination of beneficial changes and while given within an obesogenic diet, and that this has led to dramatic changes in body fat content, such as prevention of liver fat increase that is seen in the HF diet containing Cs.

MEMRI analysis following dietary supplementation indicates a significant increase in ARC SI following oligofructose-enriched In supplementation of a HF diet. Our group has previously demonstrated a significant reduction in activation in ARC SI in *ad libitum* fed compared to fasted mice (16,24). Therefore, the increase in SI we have recorded in the ARC following oligofructose-enriched In supplementation suggests an increase in hypothalamic neuronal activation similar to the fasted state. This result was surprising as our food intake data reveals a decrease in cumulative caloric intake in the HF+In group and we would therefore have expected to see a reduction in SI. However, it should be noted that the aforementioned MEMRI studies were of an acute nature and not directly comparable to the long-term dietary manipulation we have performed here. Previous data from our laboratory showed that animals receiving dietary supplementation with nondigestible, high-resistant starch, with similar reductions in body weight and adiposity, also demonstrated a trend towards increased ARC SI (7). Our animal model of dietary In supplementation exhibits numerous physiological changes in keeping with previous publications and in conjunction with the similarities to this previous chronic MEMRI study, we feel this change in ARC SI is a genuine effect.

It should be noted that, like all functional imaging techniques, changes in MEMRI SI are a reflection of net alterations in neuronal activity. The observation of an increase in SI may therefore indicate activation of either stimulatory or inhibitory neurons. Similarly, no change in SI may correspond to a lack of effect of a stimulus or it might represent a decrease in activity in one subpopulation of neurons balanced by a corresponding increase in activity in another subpopulation. Ultimately, this makes the difference in ARC signal intensity following In supplementation all the more intriguing.

The food restriction and fat depletion we have observed in HF+In animals are known to trigger multiple peripheral and central systems designed to increase energy intake (37). In accord with the trend towards increased GLP-1 we have observed here, dietary oligofructose and In supplementation has been shown to reduce circulating levels of the orexigen ghrelin while increasing levels of the anorexigenic peptide GLP-1 (8). Additional MEMRI studies by our laboratory have shown that peripheral administration of GLP-1 reduces arcuate hypothalamic activity while ghrelin increases it (15,16). These data would appear to contradict the increase in ARC SI we have observed here; however, like the aforementioned fed and fasted state studies, they are of an acute rather than long-term design. A key issue for the majority of long-term dietary or pharmacological interventions designed to combat obesity is the return to original body weight, postintervention. The mechanisms that defend body weight are likely to exist in basal appetite centers such as those found in the hypothalamus and it is therefore possible that the increase we have recorded in ARC SI represents a physiological repletion signal that manifests postintervention (38). This theory would require In supplementation to override this hypothalamic driven urge to eat, perhaps via reward-driven circuits in cortical regions of the brain (37). Of course without additional work this is purely speculation, however our data indicate that MEMRI is capable of detecting the long-term effects of dietary supplementation on hypothalamic activation.

It has been known for some time from work on alcohol metabolism that acetate can have an effect on the central nervous system and induce changes in behavior (12). It is well established that oligofructose and In feeding raises peripheral circulating acetate and propionate (10). Recent evidence from ^{11}C-labeled acetate shows that acetate gets across the blood–brain barrier (11). There is an indication that the SCFA receptor FFAR3 is present in the brain (39). Because of the restricted plasma volume we were not able to directly measure plasma SCFA. Evidence suggests that high colonic SCFA levels, which we observed in this study, lead to higher circulating concentrations (40). It is possible to hypothesize that SCFA may have direct effects on hypothalamic neuronal activation. Unfortunately the limited plasma volume from mice restricts extensive metabolic profiling. Ideally, we would have liked to measure adipocyte signals such as leptin, adiponectin, and resistin. Leptin in particular may be of interest as there is suggestion that SCFA may be involved in its release through activation of the receptor FFAR3 on adipocytes. Also to fully understand the interaction between In and its fermentation products there is a need to investigate more gut hormones; peptide YY, cholecystokinin, insulin, pancreatic polypeptide and ghrelin which is difficult with limited plasma volume.

In conclusion, our comprehensive system approach in the same animal revealed that hypercaloric feeds enriched with fermentable fibre results in reduced weight gain and an improvement in body composition and metabolic function. Our results also suggest that the mechanism behind these observations is multifactorial. There is a trend towards a higher circulating anorectic gut hormone GLP-1 in the In groups which could influence body weight. There is a shift in the microbiotia population towards a bacterial profile that increase SCFA production. We have demonstrated that oligofructose and In modulate hypothalamic neuroactivation which may be due to the combined effects of gastrointestinal hormones and SCFA.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

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DISCLOSURE

J.V.L. is an employee of BENEO-Orafti, Belgium. All the other authors declared no conflict of interest.

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