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The Impact of Oligofructose on Stimulation of Gut Hormones, Appetite Regulation, and Adiposity

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Abstract

Objective: To investigate the effect of nutrient stimulation of gut hormones by oligofructose supplementation on appetite, energy intake (EI), body weight (BW) and adiposity in overweight and obese volunteers.

Methods: In a parallel, single-blind and placebo-controlled study, 22 healthy overweight and obese volunteers were randomly allocated to receive 30 g day⁻¹ oligofructose or cellulose for 6 weeks following a 2-week run-in. Subjective appetite and side effect scores, breath hydrogen, serum short chain fatty acids (SCFAs), plasma gut hormones, glucose and insulin concentrations, EI, BW and adiposity were quantified at baseline and post-supplementation.

Results: Oligofructose increased breath hydrogen ($P < 0.0001$), late acetate concentrations ($P = 0.024$), tended to increase total area under the curve (tAUC)_{420mins} peptide YY (PYY) ($P = 0.056$) and reduced tAUC_{450mins} hunger ($P = 0.034$) and motivation to eat ($P = 0.013$) when compared with cellulose. However, there was no significant difference between the groups in other parameters although within group analyses showed an increase in glucagon-like peptide 1 (GLP-1) ($P = 0.006$) in the cellulose group and a decrease in EI during ad libitum meal in both groups.

Conclusions: Oligofructose increased plasma PYY concentrations and suppressed appetite, while cellulose increased GLP-1 concentrations. EI decreased in both groups. However, these positive effects did not translate into changes in BW or adiposity.

Introduction

Obesity is associated with numerous comorbidities including type 2 diabetes, cardiovascular disease and cancers (1). Dietary components that promote satiety and suppress energy intake could offer a public health solution to weight gain in adults. Certain dietary fibers have beneficial effects in modulating food intake and body weight (BW) (2,3). We and others have shown that inulin-type fructans supplementation can stimulate the release of anorectic gut hormones which is related to reduced appetite (4) and energy intake (5) in humans. The mechanisms behind these observations are unclear, but may be due to increased colonic fermentation resulting in greater production of short chain fatty acids (SCFAs). SCFAs are thought to modulate appetite by binding to their receptors, free fatty acid receptors 2 and 3 (6,7) triggering colonic L-cells to release anorectic gut hormones, including GLP-1 and PYY (8-10).

Oligofuctose is a fermentable carbohydrate, which is known to affect appetite (5,11), however it is unclear whether this effect is due to its fermentation in the gut or other large bowel effects. To date, there have been no studies directly compared the effect of oligofuctose on energy regulation with an inert dietary fiber. Indeed, in previous studies oligofuctose has been compared to maltodextrin, an absorbable carbohydrate as a control (5,11-13). Little is known regarding the effects of oligofuctose supplementation on body fat distribution and in particular ectopic fat content in humans. In rodents, we and others have demonstrated a consistent reduction in visceral adipose tissue (AT) and liver fat (14,15). This is of interest as increased visceral AT and liver fat accumulation have been associated with metabolic disturbances, including insulin resistance, hypertension and diabetes independent of BW (16,17).

Previous studies looking at the effects of oligofuctose supplementation on body composition did showed a significant reduction in BW in adults (12) and total fat mass in adolescents (18) when compared to control. However, these studies have employed dual energy X-ray absorptiometry, which does not allow direct measurement of individual fat depots.

Therefore, in this 8-week supplementation study, we investigated the role of oligofuctose compared to a nonfermentable fiber, cellulose on gut hormone concentrations, appetite, energy intake and assessed changes in total and regional body fat content using magnetic resonance imaging (MRI) and spectroscopy (MRS) in free living adults.

Methods

Volunteers

All volunteers gave informed consent prior to the start of the study. The study protocol was approved by the Hounslow and Hillingdon Research Ethics Committee (project registration number: 09/H0709/18) (Clinical trial number: NCT00912197) and carried out in accordance with the Declaration of Helsinki. Eligibility criteria included healthy adults with a BMI in the range 25-35 kg/m², aged 20-50 years, and non-smokers. Emotional, external and restraint scores were assessed using the Dutch Eating Behaviour Questionnaire. Exclusion criteria included chronic diseases, gastrointestinal disease, pregnancy or breastfeeding, any medication, unstable BW within the 3 months before the study, an excessive diet or physical activity regime, ingestion of prebiotic or probiotic products >three times per week, and dietary fiber intakes >25 g day⁻¹. The recruited volunteers were randomly assigned to the oligofructose or cellulose supplementation using an envelope system based on sex and gender by a member of the department who was not directly involved in this study.

Dietary fiber supplements

Oligofructose (Beneo™ P95: Orafiti, Tienen, Belgium) and cellulose were provided as white, powdered supplements in sachets each containing 10 g dietary fiber. Maltodextrin was added to the cellulose supplement to make the two supplements isoenergetic (47.4 kcal/198.6 kJ per 30 g fiber supplementation). Volunteers were instructed to take the supplement with their main meals.

Study protocol

The study design is summarized in Supporting Information Figure S1A. This was a randomized, parallel, single-blinded, controlled study. Volunteers attended five study visits, three visits for appetite measurements [Visits 1 (acclimatization visit to familiarize subjects with the study procedures), 3 (day 0) and 4 (day 56)] and two visits for MRI total body scans (visits 2 and 5). The 8-week supplementation period took place between visits 3 and 4 and included a 2-week run-in period to allow the bowel to adapt to the 30 g of dietary fiber.

Appetite study day

On each study visit, weight was measured prior to the start of the study. Subjective appetite and side effect ratings [assessed by visual analogue scales (VAS) (19)], breath hydrogen and blood samples were obtained from volunteers throughout the study session. Standardized breakfast and lunch meals were served at 0 and 240 min, respectively. Energy intake was assessed at an ad libitum

meal at 420 min using a homogenous pasta or rice based meal (each volunteer had the same meal on each occasion). Volunteers were instructed to eat until comfortably full and were left alone and undisturbed whilst eating. Food and water were weighed before and after the assessment. On post-supplementation visit (day 56), 30 g of the fiber supplements were split into two equal portions and added to the fruit juice, which accompanied the breakfast and lunch meals. The appetite study protocol is summarized in Supporting Information Figure S1B.

Estimation of colonic fermentation

Colonic fermentation activity was estimated by measuring breath hydrogen using a portable handheld monitor (Gastrolyzer, Bedfont Scientific, Kent, UK).

Blood sampling

A cannula was inserted in a forearm vein for collection of venous blood samples throughout the three study days. Blood for gut hormone analyses was collected into potassium EDTA tubes containing aprotinin (Trasylol, Bayer, Newbury, UK). Blood samples were centrifuged at 3000g at 4°C for 10 min, separated into plasma or serum and stored at -80°C until analyzed.

Free-living appetite and energy intake assessment

To estimate the impact of the supplementation in the home environment we assessed appetite sensations by VAS and energy intake by 7-day food diary at baseline and the last week of the supplementation period. Food diaries were analyzed using Dietplan6 (Forestfield Software, West Sussex, UK). During the supplementation, volunteers were contacted by investigators by phone every 2 weeks. Compliance was monitored by assessing unused sachets returned by volunteers at the end of the study.

Biochemistry

Total PYY and GLP-1 concentrations were quantified using specific and sensitive in-house radioimmunoassays as previously described (20,21). Plasma insulin concentrations were assayed using RIA kits (Millipore, MO). The sensitivity concentrations for PYY, GLP-1 and insulin were 2.5 pmol l⁻¹, 7.5 pmol l⁻¹, and 1.4 μU ml⁻¹, respectively. The intraassay variations were 4.1, 3.3, and 7.6%, respectively. Glucose, fasting insulin, cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltranspeptidase (γGT) were analyzed in the Department of Clinical Biochemistry, Hammersmith Hospital, London using an Abbott Architect

ci8200 analyzer (Abbott Diagnostics, Maidenhead, UK). Fasting glucose and insulin were used to calculate HOMA-IR and β -cell function (%B) (22).

Serum SCFA measurement

Serum SCFA concentrations at 0, 300, and 450 min were measured by gas chromatography modified from previously described methods (23,24). See Supporting Information for details.

MRI study days

Total and regional body composition and anthropometry (weight, height, waist and hip circumferences) were measured. BMI and waist:hip ratio (WHR) were then calculated. Blood for biochemical measurements were obtained after 12-h overnight fast.

MRI whole body and regional adipose tissue content

Rapid T1-weighted magnetic resonance images were obtained using a 1.5T Phillips Achiva scanner (Phillips, Best, the Netherlands), as described previously (25) (see Supporting Information). Intrahepatocellular lipids (IHCL) and pancreatic fat were measured relative to liver water content, as previously described (26). Soleus and tibialis IMCL lipid concentrations were measured relative to total muscle creatine signal, as previously described (27).

Statistical analyses

Data are presented as mean \pm standard error (SEM) unless otherwise stated. The sample size was based on power calculations that used plasma PYY concentrations as a primary outcome (4). With the estimated increase of $tAUC_{480mins}$ PYY of 5234 ± 3638 pmol min⁻¹ (mean \pm SD) based on 0.8 power to detect a significant difference ($P=0.05$, two tailed), a minimum of 18 participants were needed. To allow for dropouts, 28 volunteers were recruited. Data were checked for Gaussian distribution using D'Agostino & Pearson omnibus normality test. Nonparametric data were log transformed and geometric means with 95% confidence interval are reported. Analysis of covariance (ANCOVA) with baseline values, age, BMI, and gender as covariates was used to compare oligofructose and cellulose treatments. Within group effects were assessed using two-tailed paired t test if normally distributed and Wilcoxon signed-ranks test if the data was non-parametric. Significance was assumed as $P<0.05$. Analyses were performed using GraphPad Prism Version 5.0 (GraphPad Software, San Diego, CA) and ANCOVA was performed using SPSS 20.0 (SPSS, Chicago, IL).

Results

Volunteers

A total of 28 volunteers were recruited, 22 volunteers completed the study. Six volunteers withdrew from the study due to work and personal reasons unrelated to the supplementation. Baseline characteristics of the 22 volunteers are described in Table 1.

Supplementation compliance

Four volunteers reported side effects such as bloating, flatulence or stomach discomfort during week 3 and 4, and were allowed to continue taking two sachets per day (20 g) during this time. However, they managed to consume 30 g day⁻¹ fiber in week 5 and 6. This resulted in a compliance in the range of 60-77% in these four volunteers whilst the remaining volunteers had compliance rates between 86 and 100% leading to an overall group compliance rate of 89.8%±13.1% in the oligofructose group (n=12) and 89.7%±12.8% in the cellulose group (n=10).

Anthropometry

No significant differences were observed in any of the anthropometric measurements before or after the intervention (Table 1).

Breath hydrogen secretion

Oligofructose significantly increased breath hydrogen concentration compared to cellulose (P=0.001) (Figure 1), with a three-fold increase in tAUC_{450mins} from baseline in the oligofructose group whilst there was no significant effect of tAUC_{450mins} within the cellulose group (P=0.435).

Serum SCFA analysis

Oligofructose significantly increased acetate concentrations at 450 min (P=0.024) and showed a tendency to increase acetate concentration at 300 min (P=0.077) compared with cellulose. In addition, within group analysis demonstrated that oligofructose significantly increased fasting serum propionate (P=0.019) and butyrate (P=0.034) concentrations and tended to increase tAUC_{450mins} butyrate (P=0.073) compared to baseline. In contrast, intake of cellulose significantly reduced acetate concentration at 300 min (P=0.012) and tAUC_{450mins} (P=0.030) compared to baseline.

Hormone and glucose responses

Oligofructose significantly increased AUC_{300-420mins} PYY (P=0.042) and tended to increase tAUC_{420mins} PYY (P=0.056) when compared to cellulose (Figure 2A). In contrast, oligofructose had no significant

impact on GLP-1 concentration when compared to cellulose ($P=0.327$) (Figure 2B). Similarly, fasting plasma PYY and GLP-1 concentrations were also not significantly affected by treatments ($P=0.799$, $P=0.823$, respectively). Nevertheless, within group analysis showed that cellulose significantly increased GLP-1 $tAUC_{420mins}$, $AUC_{0-300mins}$ and $AUC_{300-420mins}$ ($P=0.006$, $P=0.021$, and $P=0.006$, respectively) compared to baseline. Oligofructose also significantly increased GLP-1 $AUC_{0-300mins}$ ($P=0.042$) compared to baseline.

There was no significant difference in glucose responses between oligofructose and cellulose treatments ($P=0.744$) (Figure 2C). However, oligofructose has a tendency to increase $tAUC_{420mins}$ and $tAUC_{300-420mins}$ glucose response when compared to the baseline ($P=0.059$ and $P=0.062$, respectively), whilst no significant effect was found within cellulose group ($P=0.993$). There was no significant difference in $tAUC_{420mins}$ insulin between treatment ($P=0.990$) or within groups ($P=0.512$ and $P=0.943$ for oligofructose and cellulose, respectively) (Figure 2D).

Energy intake assessments

No significant difference in energy intake at the ad libitum meal was observed between groups ($P=0.578$). However, oligofructose significantly reduced energy intake compared to baseline (873.16 ± 54.06 kcal to 760.19 ± 51.59 kcal, 12.9% reduction, $P=0.007$) with a similar trend in the cellulose group (867.61 ± 159.45 kcal to 725.34 ± 117.40 , 16.4% reduction, $P=0.05$).

Food diary analyses showed no significant change in energy intake between groups ($P = 0.821$) or within oligofructose or cellulose groups ($P=0.522$ and $P=0.652$, respectively). Two volunteers were under-reporters. However, omitting these volunteers from the analysis did not reveal any effect of treatments on energy intake ($P>0.05$). No significant effects of treatments on macronutrient or fiber (without supplementation) intakes were found ($P>0.05$) (data not included).

Appetite sensation ratings

Oligofructose significantly reduced hunger ($P=0.034$), motivation to eat ($P=0.013$) and desire to eat savoury food ($P=0.003$), fatty food ($P=0.013$) and salty food ($P=0.009$), but did not have a significant effect on fullness scores ($P=0.493$) when compared to cellulose (Table 3). There was no significant difference between treatments ($P=0.135$) in appetite sensation assessment during home supplementation, although there was a trend toward a reduction in hunger scores within the oligofructose group ($P=0.054$).

Gastrointestinal side effect assessments

On the appetite study day, oligofructose increased flatulence ($P=0.005$) and bloating scores ($P=0.007$) when compared to cellulose whilst no significant effect of treatments on gastrointestinal side effect scores was demonstrated during home supplementation (Table 3).

Total and regional adipose tissue content

Total and regional AT content were not affected by the treatments (Table 4). However, there was a trend for an increase of internal AT in the oligofructose group although these changes did not reach significance ($P=0.065$). Similarly, intra-abdominal AT (IAAT) within oligofructose group was significantly increased ($P=0.043$) after the supplementation, although the percentage difference between the groups was not significant ($P=0.257$).

MRS of liver, muscle, and pancreas fat

The ^1H MRS of liver, muscle and pancreas fat results are shown in Table 4. No significant differences were observed in any on the ectopic fat depots measured.

Biochemical analysis

No significant differences were found in any of the biochemical measurements (AST, ALT, γ GT, glucose, insulin, HOMA-IR, HOMA % B and lipid profiles) (Supporting Information Table S1).

Discussion

The effects of fermentable carbohydrates, such as oligofructose, on appetite and adiposity in humans are poorly understood. In this study, we evaluated the effect of 8 weeks high dose oligofructose supplementation on gut hormones, appetite, weight loss, and body fat distribution.

The current study confirmed the results of previous studies, that oligofructose significantly reduces subjective hunger, increases breath hydrogen (5,11) and plasma PYY concentrations (12,13). However, energy intake at ad libitum meal in the oligofructose group was not significantly different from the cellulose group although it was decreased within the groups. There was also no effect of treatment on BW and overall body fat content. In this study we controlled for material entering the colon by giving cellulose, a nonfermentable fiber source which did not increase breath hydrogen suggesting minimal fermentation. Surprisingly, we did observe an increase in GLP-1 concentration and a decrease in food intake in the cellulose group, suggesting despite the apparent inert nature of cellulose, it has an effect on GLP-1 release. Insoluble fiber like cellulose potentially stimulates GLP-1 secretion by increasing the time it takes fiber to reach the distal small intestine (28). However, this increase did not make an impact on insulin release. This raises the possibility that the two fibers have different effects on gut hormones.

Rodent studies suggest that stimulation of colonic fermentation by inulin-type fructans increase GLP-1₇₋₃₆ and PYY release from the intestinal L-cells (8,29). In our study, intake of oligofructose led to a 3-fold increase in breath hydrogen concentrations, a marker of colonic fermentation, and a significant increase in serum SCFAs. These observations were associated with a significant increase in PYY, but no significant modulation in GLP-1. There are conflicting reports regarding the effect of oligofructose on circulating GLP-1 concentrations in human studies; some have shown that oligofructose significantly increases GLP-1 concentrations (11,13,30) whilst others did not (4,12). It may be that fermentation of inulin-type fructans in the human colon causes a differential release of PYY.

The increase in breath hydrogen, SCFAs and PYY also coincided with increases in fullness and decreases in hunger, motivation to eat and energy intake at the ad libitum meal. However, this was not translated into an effect on BW. This may be due to hedonic drive to maintain energy homeostasis in the long term overriding the anorectic signals from the gut hormones. It is possible that although oligofructose significantly raised plasma PYY concentrations, they did not reach the level of plasma concentrations observed following PYY infusions which resulted in a significant decrease in food intake [current study peak concentration 40 pmol l⁻¹ vs. 60 pmol l⁻¹ (31)].

Previous studies have compared oligofructose to readily digestible carbohydrates (maltodextrin) (5,11-13). In this study, we aimed to evaluate the effect of oligofructose when compared to cellulose, a different fiber. Cellulose mainly acts as a bulking agent (32). However, recent reports suggest that it can be fermented by gut microbiota, but to a much lesser extent than oligofructose (33). However, despite the different physicochemical properties of cellulose and oligofructose, they may have promoted satiety signals at different parts of the gut, thus resulting in little or no difference in effects on appetite, BW and body fat between the treatments.

Previous studies using oligofructose as a dietary supplement have shown a reduction in BW and overall body fat content when compared to control treatments (12,18). However, body fat in these studies was only reported as a total adiposity and therefore the role of oligofructose on body fat distribution (total, abdominal, and regional adiposity) remains unclear. In the present study, oligofructose supplementation had no significant effect on total body fat content or BW when compared to cellulose. Overall, it appeared that oligofructose altered body fat distribution, whilst only IAAT achieved significance, there was a consistent trend for an increase in internal body fat stores following OFS supplementation. This is at odds with previous rodent studies which have reported reduced internal fat in similar interventions. Although oligofructose had no significant effect on ectopic fat content compared with cellulose, our investigation showed a tendency to reduce IHCL within the oligofructose group. However, both groups had mean baseline IHCL concentrations within the normal range (34), making it more difficult to achieve a significant reduction in what were already relatively low levels of liver fat. This may also in part explain why no significant changes in biochemical markers of liver function (AST, ALT, and cGT concentrations) were found in this study. Additionally, lack of change in ectopic fat accumulation in either the soleus and tibialis muscles or pancreas could possibly be related to no change in HOMA-IR and HOMA-B, biomarkers for insulin resistance.

The nonsignificant changes in body fat content and distribution in the current study may reflect the relatively small sample size, giving us insufficient power to detect subtle differences between treatments. Furthermore, the 8-week period of supplementation may not be long enough for oligofructose to exert its effects despite the relatively high dose of 30 g oligofructose. Indeed, other short term studies on fermentable carbohydrates have reported similar negative results (35,36). Thus, it is possible that fermentable fibers need a longer period of supplementation to affect BW and body fat.

In summary, the results of this study suggest that oligofructose potentially has a short term effect on appetite possibly modulated through colonic fermentation and the anorectic gut hormone, PYY. Although not significant, the slightly reduced IHCL and cGT concentrations from baseline may suggest that the beneficial effect of oligofructose supplementation perhaps is by protecting the liver against fat accumulation. However, the increases observed in internal fat depots suggest that future studies would be needed to determine whether any beneficial changes to appetite outweigh possible negative changes in body fat distribution. Also, we report that the control cellulose increase GLP-1 release without a measurable increase in fermentation.

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TABLE 1: Volunteers characteristics at baseline and anthropometric measurements at baseline and post-supplementation^a.

Anthropometric characteristics	Oligofructose		Cellulose		P value
	(n=12)	Range	(n=10)	Range	
Females/males (n)	9/3	-	7/3	-	-
Age (y)	36.5 ± 2.2	21 - 49	28.7 ± 2.3	20 - 47	0.025 ^b
Emotional score	2.3 ± 0.4	1.0 - 4.8	2.0 ± 0.2	1.3 - 2.9	0.830 ^b
External score	2.9 ± 0.2	1.5 - 4.1	2.7 ± 0.2	1.5 - 4.1	0.625 ^b
Restraint score	2.1 ± 0.2	1.2 - 3.1	2.2 ± 0.2	1.2 - 3.1	0.647 ^b
BW (kg) (day 0)	83.7 ± 4.9	59.4 - 121.2	86.0 ± 4.4	65.9 - 106.4	0.742 ^b
BW (kg) (day 56)	84.1 ± 4.8	58.7 - 117.5	86.3 ± 4.5	66.3 - 108.2	0.715 ^c
BMI (kg/m ²) (day 0)	29.7 ± 1.0	25.0 - 34.6	31.1 ± 1.1	26.0 - 35.0	0.364 ^b
BMI (kg/m ²) (day 56)	29.9 ± 1.1	24.8 - 35.0	31.2 ± 1.1	25.8 - 35.0	0.715 ^c
WHR (day 0)	0.86 ± 0.03	0.68 - 1.0	0.82 ± 0.02	0.73 - 0.94	0.270 ^b
WHR (day 56)	0.88 ± 0.02	0.77 - 0.98	0.83 ± 0.03	0.75 - 0.98	0.521 ^c

^aData are expressed in means ± SEM.

^bTest for difference between groups for baseline measurement was performed by unpaired *t*-test.

^cTest for difference between groups were conducted by ANCOVA, with baseline, age and gender as covariates,

**P* < 0.05

TABLE 2: SCFA concentrations and tAUC_{450mins} measured on the baseline (Day 0) and post-supplementation (Day 56) appetite study day ¹

Type of SCFA		Timepoint (mins)	Oligofructose (n=12)		Cellulose (n=10)	
			Day 0	Day 56	Day 0	Day 56
Total fasting	μM	0	82.6 ± 2.2	83.7 ± 2.0	80.7 ± 3.1	83.4 ± 2.9
Total postprandial	μM	300	87.0 ± 2.2	89.2 ± 2.1	91.4 ± 3.4	84.6 ± 2.9
	μM	450	82.4 ± 2.5	85.6 ± 2.4	79.9 ± 2.6	77.8 ± 2.2
Acetate	μM x min	tAUC	35615.1 ± 776.1	36434.3 ± 730.3	36086.7 ± 1131.9	34951.8 ± 1105.7
		0	77.4 ± 1.8	77.3 ± 5.8	75.0 ± 2.9	77.5 ± 2.5
		300	81.1 ± 2.0	83.7 ± 2.1	85.2 ± 3.2	78.6 ± 2.6 ^b
		450	76.8 ± 2.5	81.3 ± 2.1 ^c	75.1 ± 2.4	72.8 ± 1.8
Propionate	μM x min	tAUC	35601.8 ± 732.1	36310.6 ± 746.2	36023.7 ± 1144.3	34706.2 ± 1058.9 ^b
		0	4.8 ± 0.5	5.4 ± 0.6 ^b	5.1 ± 0.6	5.0 ± 0.5
		300	5.3 ± 0.5	5.4 ± 0.6	5.2 ± 0.6	5.2 ± 0.5
		450	5.3 ± 0.5	5.4 ± 0.6	5.1 ± 0.5	5.4 ± 0.6
Butyrate	μM x min	tAUC	2311.7 ± 209.1	2405.0 ± 237.4	2308.6 ± 263.5	2323.3 ± 227.7
		0	0.4 ± 0.1	0.8 ± 0.1 ^b	0.6 ± 0.2	0.9 ± 0.1
		300	0.6 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.2
		450	0.5 ± 0.1	0.9 ± 0.1	0.5 ± 0.2	0.7 ± 0.2
	μM x min	tAUC	242.6 ± 29.4	340.8 ± 42.4	323.4 ± 52.2	358.2 ± 53.1

^aData are expressed in means ± SEM

^b Significantly different from Day 0, **P*< 0.05

^cSignificantly different from cellulose group, **P*< 0.05

TABLE 3: Subjective appetite sensations measured on the baseline (Day 0) and post-supplementation (Day 56) appetite study day and supplementation period, baseline (Day -8 to -1) and during supplementation (Day 49 -55)^a

Appetite sensation	Oligofructose (n=12)		Cellulose (n=10)		P Value ^b
	Day 0	Day 56	Day 0	Day 56	
Appetite study day					
Hunger (cm min)	2053.9 ± 308.7	1387.3 ± 263.2 ^c	1850.6 ± 395.3	1838.6 ± 315.9	0.034
Fullness (cm min)	1790.8 ± 243.7	2095.3 ± 249.3 ^c	1615.1 ± 314.5	1805.3 ± 254.7	0.493
Motivation to eat (cm min)	2059.6 ± 294.5	1412.5 ± 229.2 ^c	1946.4 ± 367.6	1847.4 ± 313.4	0.013
Desire to eat sweet food (cm min)	1759.5 ± 372.2	1232.8 ± 361.3 ^c	2022.8 ± 444.9	1904.3 ± 364.1	0.342
Desire to eat savoury food (cm min)	1658.0 ± 365.3	1090.6 ± 251.1 ^c	1534.7 ± 445.3	1757.7 ± 420.0	0.003
Desire to eat fatty food (cm min)	1401.9 ± 324.8	566.3 ± 191.2 ^c	1022.4 ± 380.8	1092.5 ± 320.9	0.013
Desire to eat salty food (cm min)	1617.4 ± 369.6	880.8 ± 238.8 ^c	1035.7 ± 350.6	1322.0 ± 341.4	0.009
Bloating (cm min) ^d	15.1 (1.0 – 218.6)	51.6 (2.7 – 985.8) ^c	105.2 (21.6 – 512.8)	14.1 (0.6 – 363.4)	0.007
Stomach discomfort (cm min) ^d	72.6 (19.4 – 271.5)	4.0 (0.2 – 75.4) ^c	43.3 (11.6 – 161.0)	10.6 (1.1 – 106.1)	0.011
Flatulence (cm min) ^d	11.1 (0.9 – 132.4)	57.6 (7.0 – 470.5) ^c	32.8 (3.8 – 280.5)	5.9 (0.4 – 99.1)	0.005
Diarrhoea (cm min) ^d	1.2 (0.1 – 9.8)	0.5 (0.1 – 3.9)	5.1 (1.1 – 24.0)	0.5 (0.1 – 5.1) ^b	0.896
Sickness (cm min) ^d	14.4 (2.5 – 81.8)	1.9 (0.2 – 20.9) ^c	19.4 (5.6 – 67.8)	2.4 (0.2 – 37.9)	0.613
Home supplementation period					
Hunger (cm)	4.8 ± 0.9	3.6 ± 0.8	3.6 ± 0.8	4.1 ± 0.6	0.135
Fullness (cm)	4.7 ± 0.5	4.2 ± 0.8	4.2 ± 0.8	4.1 ± 0.6	0.688
Bloating (cm) ^d	0.9 (0.2 – 1.5)	2.3 (0.6 – 3.9)	0.4 (0.1 – 1.2)	0.4 (0.1 – 1.2)	0.974
Stomach discomfort (cm) ^d	0.5 (0.2 – 1.2)	0.4 (0.1 – 1.2)	0.2 (0.1 – 0.8)	0.2 (0.1 – 0.8)	0.868
Flatulence (cm) ^d	0.9 (0.4 – 1.5)	1.7 (0.3 – 3.2)	0.3 (0.1 – 1.1)	0.3 (0.1 – 1.3)	0.224
Diarrhoea (cm) ^d	0.1 (0.04 – 0.2)	0.1 (0.04 – 0.5)	0.01 (0.03 – 0.3)	0.1 (0.04 – 0.4)	0.837
Sickness (cm) ^d	0.1 (0.05 – 0.3)	0.1 (0.05 – 0.4)	0.1 (0.04 – 0.4)	0.2 (0.04 – 0.6)	0.760

^aData are expressed in means ± SEM for normal distribution and geometric mean (95% confidence interval) for non-normally distributed data

^b Significantly different from Day 0, **P* < 0.05 (Paired *t*-test)

^cTest for difference between groups were conducted by ANCOVA, with baseline, age and gender as covariates, **P* < 0.05

^dData analysis was performed on log transformed values. Values presented as anti-log of the mean of log-transformed values.

TABLE 4: Total and regional body adipose tissue before and after intervention in the treatment groups^a

AT (L)	Group	Pre	Post	P value^b
TAT	OFS	36.3±2.8	37.0±2.8	0.858
	CEL	38.4±3.7	38.7±3.8	
SAT	OFS	30.4±2.7	30.6±2.7	0.914
	CEL	33.8±3.4	34.1±3.4	
IAT	OFS	5.9±0.8	6.4±0.8	0.489
	CEL	4.6±0.4	4.5±0.5	
IHCL^c	OFS	1.9 (0.6-6.0)	1.4 (0.4-4.9)	0.814
	CEL	0.7 (0.4-1.2)	0.6 (0.3-1.1)	
S-IMCL^c	OFS	10.7 (6.8-17.0)	12.0 (8.1-18.1)	0.883
	CEL	12.6 (7.2-22.1)	12.0 (7.4-19.2)	
T-IMCL	OFS	7.2±1.0	6.5±1.1	0.366
	CEL	7.0±0.9	7.1±0.8	
Pancreas^c	OFS	2.9 (2.0-4.0)	2.8 (1.8-4.2)	0.719
	CEL	1.6 (1.1-2.3)	1.8 (1.3-2.4)	
ASAT	OFS	9.5±1.0	9.5±1.0	0.194
	CEL	10.2±1.1	10.4±1.2	
NASAT	OFS	20.9±1.8	21.1±1.8	0.753
	CEL	23.6±2.4	23.7±2.4	
IAAT	OFS	3.1±0.5	3.3±0.5	0.257
	CEL	2.1±0.2	2.1±0.3	
NAIAT^c	OFS	2.7 (2.1-3.4)	2.9 (2.3-3.7)	0.308
	CEL	2.4 (2.0-3.0)	2.3 (1.8-3.0)	
IAT:SAT ratio^c	OFS	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.263
	CEL	0.1 (0.1-0.2)	0.1 (0.1-0.2)	
IAAT:ASAT ratio^c	OFS	0.3 (0.2-0.5)	0.3 (0.2-0.5)	0.794
	CEL	0.2 (0.2-0.3)	0.2 (0.1-0.3)	

^aData are expressed in Mean±SEM for normally distributed and geometric mean (95% confidence interval) for non-normally distributed.

^bDifference between groups were analysed by ANCOVA, with baseline, age and gender as covariates, *P<0.05.

^cData were log transformed for analysis.

Abbreviations: ASAT: abdominal subcutaneous adipose tissue, CEL: cellulose group (n510), IAAT: intra-abdominal adipose tissue, IAT: internal adipose tissue, IHCL: intrahepatocellularlipid, NAIAT: non-abdominal internal adipose tissue, NASAT: nonabdominal subcutaneous adipose tissue, OFS: oligofructose group (n512), SAT: subcutaneous adipose tissue, S-IMCL: soleus-intramyo cellular lipid, TAT: total adipose tissue, T-IMCL: tibialis-intramyo cellular lipid.

Figure Captions

Figure 1: Mean (\pm SEM) (ppm) and $tAUC_{450min}$ (ppm*min) of breath hydrogen concentrations on appetite study day at baseline (day 0) and post-supplementation (day 56), oligofructose (n=12) and cellulose (n=10). Inset: the $tAUC_{450mins}$ breath hydrogen concentrations in the oligofructose group was significantly higher compared to cellulose group on post-supplementation day ($P = 0.001$, ANCOVA).

Figure 2: Mean (\pm SEM) concentrations and $tAUC_{420mins}$ of plasma total peptide YY (PYY) (A), total glucagon-like-peptide 1 (GLP-1) (B), glucose (C) and insulin (D) on appetite study day at baseline (day 0) and following oligofructose and cellulose supplementation on post-supplementation (day 56), oligofructose (n=12) and cellulose (n=10).

Figure 1

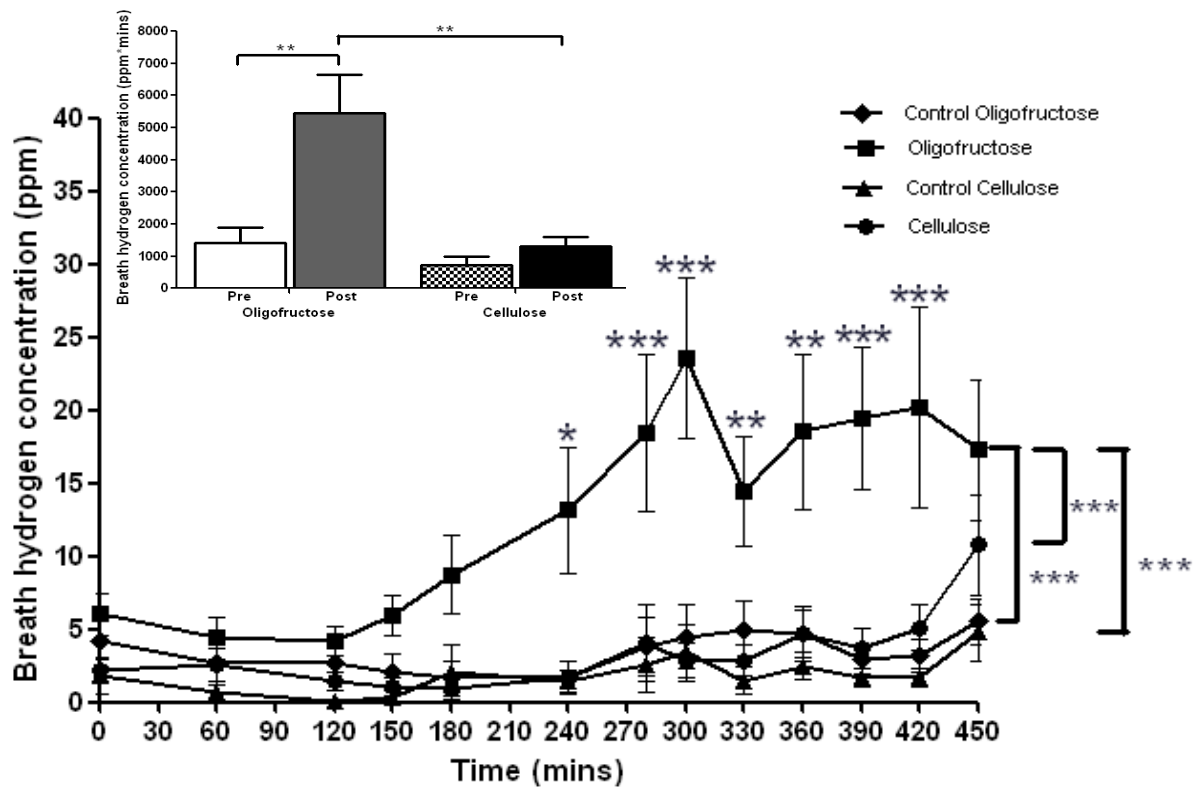
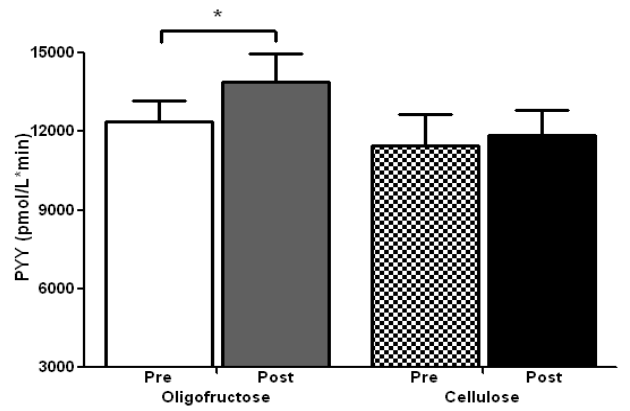
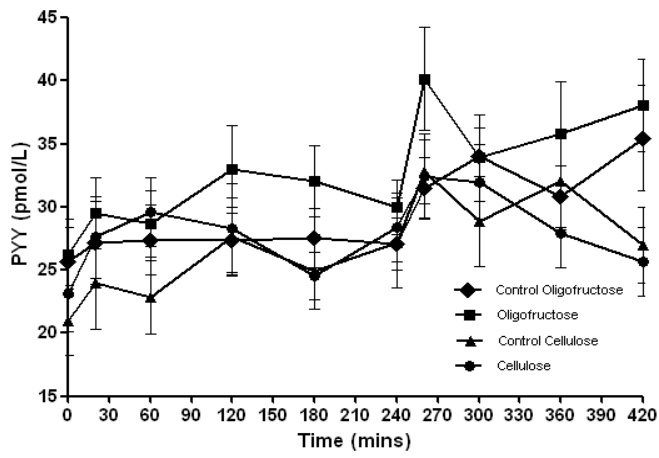
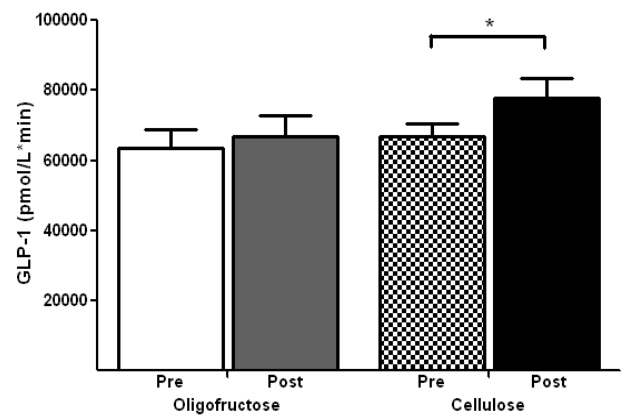
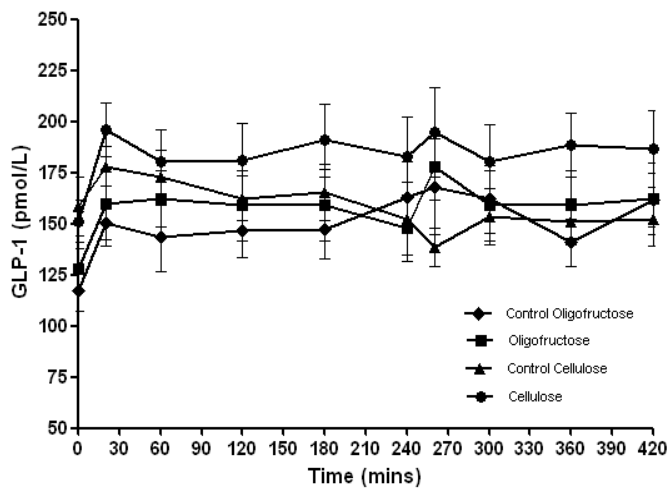


Figure 2

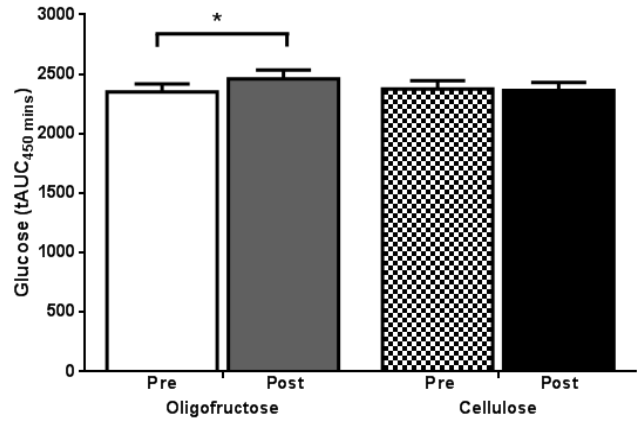
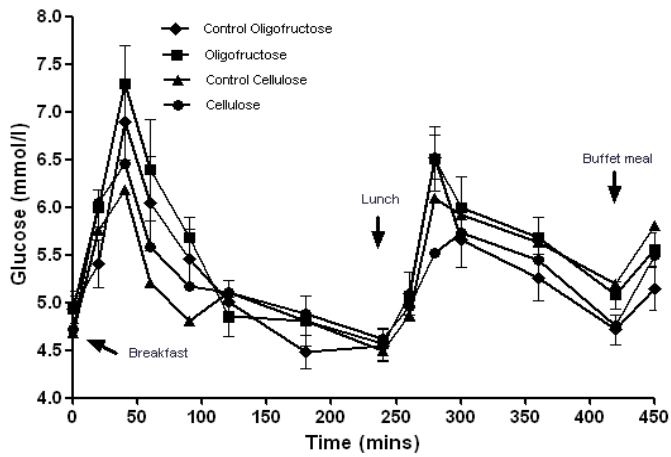
(A) Plasma PYY



(B) Plasma GLP-1



(C) Plasma Glucose



(D) Plasma Insulin

