

L-rhamnose as a source of colonic propionate inhibits insulin secretion but does not influence measures of appetite or food intake



Julia Darzi^{a,*}, Gary S. Frost^b, Jonathan R. Swann^c, Adele Costabile^d,
M. Denise Robertson^e

^a Diabetes and Nutritional Sciences Division, School of Medicine, King's College London, Franklin-Wilkins Building, London SE1 9NH, United Kingdom

^b Faculty of Medicine, Nutrition and Dietetics Research Group, Division of Diabetes, Endocrinology and Metabolism, Department of Investigative Medicine, Imperial College London, London W12 0NN, United Kingdom

^c Division of Computational and Systems Medicine, Department of Surgery and Cancer, Imperial College London, London SW7 2AZ, United Kingdom

^d Health Sciences Research Centre, Whitelands College, University of Roehampton, London SW15 5PU, United Kingdom

^e Nutritional Sciences, University of Surrey, Leggett Building, Guildford, Surrey GU2 7WG, United Kingdom

ARTICLE INFO

Article history:

Received 19 August 2015

Received in revised form

4 December 2015

Accepted 14 December 2015

Available online 17 December 2015

Keywords:

Appetite

Satiety

Postprandial insulin

Inulin-type fructans

Short-chain fatty acids

ABSTRACT

Activation of free fatty acid receptor (FFAR)2 and FFAR3 via colonic short-chain fatty acids, particularly propionate, are postulated to explain observed inverse associations between dietary fiber intake and body weight. Propionate is reported as the predominant colonic fermentation product from L-rhamnose, a natural monosaccharide that resists digestion and absorption reaching the colon intact, while effects of long-chain inulin on appetite have not been extensively investigated. In this single-blind randomized crossover study, healthy unrestrained eaters ($n = 13$) ingested 25.5 g/d L-rhamnose, 22.4 g/d inulin or no supplement (control) alongside a standardized breakfast and lunch, following a 6-d run-in to investigate if appetite was inhibited. Postprandial qualitative appetite, breath hydrogen, and plasma glucose, insulin, triglycerides and non-esterified fatty acids were assessed for 420 min, then an *ad libitum* meal was provided. Significant treatment \times time effects were found for postprandial insulin ($P = 0.009$) and non-esterified fatty acids ($P = 0.046$) with a significantly lower insulin response for L-rhamnose ($P = 0.023$) than control. No differences between treatments were found for quantitative and qualitative appetite measures, although significant treatment \times time effects for meal desire ($P = 0.008$) and desire to eat sweet ($P = 0.036$) were found. Breath hydrogen was significantly higher with inulin ($P = 0.001$) and L-rhamnose ($P = 0.009$) than control, indicating colonic fermentation. These findings suggest L-rhamnose may inhibit postprandial insulin secretion, however neither L-rhamnose or inulin influenced appetite.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Inverse associations between dietary fiber intake and body weight (Du et al., 2010; Howarth, Huang, Roberts, & McCrory, 2005), hunger and energy intake (EI) following non-digestible carbohydrate ingestion in randomized controlled trials (Wanders et al., 2011), indicate dietary fiber and other non-digestible carbohydrates may have a role in the prevention and treatment of

obesity. Postulated mechanisms include an increased viscosity of intestinal contents (Kristensen & Jensen, 2011), a reduced energy density due to the bulking effect of non-digestible carbohydrates (Burton-Freeman, 2000), and an inhibition of EI arising from effects of non-digestible carbohydrate on satiation and satiety (Burton-Freeman, 2000), possibly mediated by actions of colon derived short-chain fatty acids (SCFA).

Physiological serum SCFA concentrations are low, in the region of 1, 2 and 65 $\mu\text{mol/L}$ for fasting serum butyrate, propionate and acetate (Fernandes, Vogt, & Wolever, 2011). Postprandially SCFA concentrations appear to increase significantly in response to ingestion of some non-digestible carbohydrates including resistant starch (Robertson, Bickerton, Dennis, Vidal, & Frayn, 2005). Physiological SCFA concentrations have been shown to activate two G-protein coupled receptors, free fatty acid receptor (FFAR) 2 and

Abbreviations: AUC, Area under curve; EI, Energy intake; FFAR, Free fatty acid receptor; GLP-1, Glucagon-like receptor-1; HOMA, Homeostasis Assessment Model; iAUC, Incremental area under curve; L-Rha, L-rhamnose; PYY, Peptide YY; SCFA, Short-chain fatty acid; VAS, Visual analogue scale.

* Corresponding author.

E-mail address: julia.darzi@kcl.ac.uk (J. Darzi).

FFAR3 (Brown et al., 2003; Le Poul et al., 2003), with propionate reported as the most potent agonist (Le Poul et al., 2003). FFAR2 and FFAR3 are co-localized in colonic enteroendocrine L-cells with peptide YY (PYY) and glucagon-like peptide 1 (GLP-1) (Karaki et al., 2006; Karaki et al., 2008; Tazoe et al., 2009), both hormones which are postulated to play roles in the physiological regulation of appetite (Hussain & Bloom, 2013; Lean & Malkova, 2015). *In vivo* administration of SCFA increases plasma PYY in rats (Cherbut et al., 1998; Psichas et al., 2015) and pigs (Cuhe, Cuber, & Malbert, 2000), and of propionate increases GLP-1 and PYY via FFAR2 activation in rodents (Psichas et al., 2015). *In vitro* and *in vivo* evidence in rodents further indicates SFCA-induced FFAR2 and FFAR3 activation upregulates leptin expression in adipose tissue (Covington, Briscoe, Brown, & Jayawickreme, 2006; Xiong et al., 2004). Thus SCFA, particularly propionate, may be postulated to influence energy homeostasis and insulin secretion.

L-Rhamnose (L-Rha), a natural monosaccharide that resists digestion and absorption reaching the colon intact (Vogt, Pencharz, & Wolever, 2004b), shows promise as a suitable candidate to investigate effects on colonic propionate on appetite. Propionate is reported as the primary SCFA produced during fermentation of L-Rha *in vitro* (Fernandes, Rao, & Wolever, 2000), and L-Rha ingestion increased serum propionate concentrations in humans acutely (Vogt et al., 2004b) and chronically (Vogt, Ishii-Schrade, Pencharz, & Wolever, 2004a). Effects of L-Rha ingestion on metabolic response have been previously investigated (Vogt et al., 2004a, b); albeit not extensively, however effects on appetite have not.

Effects of supplementing with inulin-type fructans on appetite are more extensively investigated; however, results are contradictory due to variable dosages, differing types of inulin-type fructans and limitations in study design (reviewed by (Darzi, Frost, & Robertson, 2011)). While a number of studies have investigated effects on appetite of short-chain inulin-type fructans, also termed oligofructose and fructooligosaccharides (for example Hess, Birkett, Thomas, & Slavin, 2011; Parnell & Reimer, 2009; Pedersen et al., 2013; Peters, Boers, Haddeman, Melnikov, & Qvyjt, 2009; Verhoef, Meyer, & Westerterp, 2011), few investigations of long-chain inulin effects exist (Archer, Johnson, Devereux, & Baxter, 2004; Karalus et al., 2012; Tarini & Wolever, 2010). The present study therefore aimed to investigate the acute effects of providing L-Rha or long-chain inulin, following a 6-d run-in, on postprandial appetite and metabolite concentrations compared to control (no supplement).

2. Methods

2.1. Participants

Healthy, non-smoking unrestrained eaters 18–55 y were recruited via e-mail advertisement at the University of Surrey and attended the Clinical Investigation Unit (CIU) in a fasted state for screening. Inclusion criteria were BMI between 19 and 26 kg/m², fasting blood glucose <6.0 mmol/l, weight stable for at least 3 months, non-smoker and reported habitual alcohol intake ≤20 units. Exclusion criteria included following a weight reducing diet, presence of gastrointestinal, endocrine or cardiovascular disorders, history of depression, eating disorders or substance abuse, pregnancy or lactation, taking regular medication (except birth control medication), and high dietary restraint (score ≥3.5 on the Dutch Eating Behaviour Questionnaire restraint scale (Van Strien, Frijters, Bergers, & Defares, 1986)). The study was conducted according to the Declaration of Helsinki and all procedures involving human participants were approved by the University of Surrey Ethics Committee (Ref: EC/2008/53/FHMS). Participants gave written informed consent.

2.2. Study protocol

This single-blind 3-way randomized crossover study was conducted from September 2008 to May 2009. Participants commenced 1-wk study periods during which L-Rha, long-chain inulin or control (no supplement) were consumed an order randomly assigned using www.randomizer.org. The condition randomized to was concealed by providing participants with ready prepared jelly (Jell-O) and mousse containing the supplement or control (no supplement) to disguise the treatment. Each study period comprised a 6-d run-in with a study day at the CIU on Day 7 and were separated by a washout period of ≥1-wk. Prior to commencing the study, all participants who had not previously participated in an appetite study attended an initial study morning at the CIU to familiarise them with the techniques being used. To control for effects of hormonal variations throughout the menstrual cycle (Asarian & Geary, 2013), female participants not using birth control medication attended the study day at approximately the same point of the menstrual cycle for each study day during the mid-follicular phase (between days 8 and 12). Participants were informed that the study aimed to compare the effects of two fiber supplements compared to a placebo (no fiber) on appetite and metabolic response.

2.3. Six day run-in

The supplement dosages used were based on the reported dose used in previous investigations of L-Rha (Vogt, Ishii-Schrade, Pencharz, Jones, & Wolever, 2006; Vogt, et al., 2004a, b), with the equivalent inulin dose calculated to be matched by pentose/hexose equivalents. The target dose of 25.5 and 22.4 g/d for L-Rha and inulin was reached by Day 4, increasing from one-third, half and two-thirds target dose during Days 1–3. The supplement (inulin or L-Rha) or control (no supplement) was provided within two portions of jelly (Hartleys Sugar Free Jelly) during run-in to be consumed alongside participants' usual diet. Sugar free jelly was chosen as the vehicle to provide the supplement, as it did not contribute greatly to the EI, it disguised the supplement or control, and made it easy for the participant to consume the supplement, as no additional preparation was required. The jellies were collected by or were dropped off to participants every few days. Compliance, gastrointestinal symptoms and the taste of the jellies were assessed using a daily monitoring diary, and a 4-day food diary was completed from Days 3–6 using household measures. Gastrointestinal symptoms (stomach pain, diarrhoea, constipation, belching, flatulence, nausea, acid regurgitation, heartburn and bloating) were monitored on a five point scale (1: none, 3: moderate, 5: debilitating), and taste was assessed on a nine point Likert Scale.

2.4. Study day (Day 7)

Participants arrived at the CIU in a fasted state after consuming a standard low fiber meal the previous evening and avoiding alcohol and unaccustomed exercise for 24-h. Participants were required to stay in the CIU for the entire study duration and water was provided *ad libitum*. Upon arrival anthropometric measurements were taken and an intravenous cannula was inserted into an antecubital vein. Two fasting blood samples were taken 30 min and 5 min before breakfast, and hydrogen concentrations in expired breath were measured using a Gastrolyser 2 portable hand held breath hydrogen monitor (Bedfont Scientific Ltd, Rochester, UK). Following each baseline blood and breath sample appetite was subjectively assessed by 100 mm visual analogue scale (VAS) questionnaires for fullness, hunger, prospective food consumption, desire to eat meal/snack/sweet/savoury/salty/fatty and nausea as previously

described (Flint, Raben, Blundell, & A, 2000). Gastrointestinal symptoms (flatulence, diarrhoea, bloating, belch/burp, stomach discomfort, urge to defecate and heartburn) were also assessed by 100 mm VAS following the first baseline blood sample.

A standard mixed breakfast and lunch (described below) were provided at $t = 0$ min and $t = 180$ min, and consumed within 15 min. A mousse was provided alongside breakfast and lunch containing two-thirds and one-third of the daily target supplement dose, respectively, or no supplement (control). The taste of the mousse was rated after each meal using 100 mm VAS. Following each meal blood was sampled every 15 min for the first hour, then half-hourly. VAS were completed after blood samples to assess appetite half-hourly and to assess gastrointestinal symptoms hourly. Breath was sampled hourly for the first hour and half-hourly thereafter.

The cannula was removed at $t = 420$ min (240 min following lunch) after which participants were seated in individual booths and served an *ad libitum* homogenous pasta meal in a quantity exceeding usual portion sizes. They were instructed to eat until they were “comfortably full”, as previously conducted by our group (Bodinham, Frost, & Robertson, 2010; Darzi, Frost, & Robertson, 2012). Participants were then free to leave and asked to complete a food diary for the remainder of the day which, in combination with intake at breakfast, lunch and the *ad libitum* test meal, was used to determine overall 24 h intake. Dietary analysis was performed using WinDiets Professional Version 2005 (Robert Gordon University, Aberdeen, UK).

2.5. Test products and study day test meals

The supplements used were Orafit Beneo Inulin HP (DKSH Great Britain Limited, Wimbledon, UK), a high degree of polymerisation inulin (average degree of polymerisation ≥ 23), and food grade 98% L-(+)-Rhamnose Monohydrate (Vitanutrition Ltd, Co Dublin, Ireland). During the control leg, only the carrier product was consumed.

On study days the supplement under investigation was incorporated into a mousse (Angel Delight, Premier Foods Group, made using semi-skimmed milk), as used previously by our group (Bodinham et al., 2010). Two-thirds of the daily dose (16.8g L-Rha or 14.9 g inulin HP) was provided at breakfast, and one-third (8.4 g L-Rha or 7.5 g inulin HP) was provided at lunch. For control, the mousse with no added supplement was provided. To enhance compliance, participants were given a choice of four mousse flavours and were given the same flavour on each study day.

The standard mixed breakfast supplied on the study day comprised croissants (66 g) with strawberry or plum jam (28 g), the supplement-containing mousse and either water (250 g) or sugar-free cordial (42 g cordial and 208 g water). The same jam and drink was on each study day. The breakfast supplied 2074 kJ, 10.5 g CHO, 36.4 g protein and 21.1 g fat when made using strawberry jam and chocolate mousse.

The standard study day lunch comprised ham, chicken or cheese sandwiches, crisps, the supplement-containing mousse and either water or sugar-free cordial. The same sandwich filling and drink was supplied on each study day. On the first study day participants were provided eight sandwich quarters and 20 g crisps and were asked to consume at least 4 full sandwich quarters and as many crisps as they would like. On subsequent study days participants were required to consume the same number of sandwiches and amount of crisps as consumed on the first study occasion, as previously reported (Weststrate & van Amelsvoort, 1993) and used by our group (Bodinham et al., 2010).

All food ingredients were weighed to the nearest 1 g except the Angel Delight and non-digestible carbohydrate supplements which

were weighed to the nearest 0.1 g.

2.6. Ad libitum test meal

The *ad libitum* test meal comprised a homogenous pasta dish made following a standard recipe of Tesco Fusilli Pasta Twists (400 g dry weight) mixed with Ragu Original Pasta Sauce (500 g), Tesco Mild Cheddar (100 g) and Tesco Vegetable Oil (30 g). The dish supplied 9750 kJ, 81.5 g protein, 339.1 g carbohydrate, 70.0 g fat and 15.9 g fiber and had a mean weight of 1520 ± 53 g and energy density of 6.4 ± 0.2 kJ/g. The weight and energy density varied due to differing amounts of water absorbed by the pasta during cooking which was accounted for when calculating EI. The dish was weighed before and after serving to determine intake.

2.7. Biochemistry

Venous blood samples were centrifuged at 1750 g for 10 min and plasma aliquots were stored at -20 °C until analysis. Samples were batch analysed with samples from the same participant in the same batch to minimise inter-assay variability. Plasma glucose, TG, non-esterified fatty acids (NEFA) and total and HDL cholesterol concentrations were measured using commercial kits for the ILAB 650 analyzer (Instrumentation Laboratory, Milan, Italy), with an inter-assay CV of <2%. Plasma insulin concentrations were analyzed by radioimmunoassay using a commercial kit (Millipore, St. Charles, Missouri), with an inter- and intra-assay CV <10%. Serum SCFA were analyzed by gas chromatography as previously described (Bodinham et al., 2014; Vogt et al., 2004a, b).

2.8. Insulin sensitivity

Insulin sensitivity parameters were assessed on the study day (Day 7). Fasting insulin sensitivity was assessed by Homeostasis Assessment Model (HOMA) using the HOMA2 Calculator Version 2.2 (University of Oxford, Oxford, UK) to estimate steady state β -cell function, insulin sensitivity and insulin resistance from fasting plasma glucose and insulin concentrations as previously described (Levy, Matthews, & Hermans, 1998; Matthews et al., 1985; Wallace, Levy, & Matthews, 2004). Postprandial insulin sensitivity was assessed using the minimal model index method as described by (Caumo, Bergman, & Cobelli, 2000).

2.9. Calculations and statistical analysis

Area under curve (AUC) for postprandial data (appetite and gastrointestinal VAS ratings, plasma metabolites and SCFA, and breath hydrogen) was calculated by the trapezoidal rule and incremental AUC (iAUC) was also determined to allow for baseline concentration differences. Statistical analyses were conducted using SPSS for Windows (version 21, SPSS Inc, Chicago, IL). Normality was tested using the Kolmogorov–Smirnov test. Differences in the effects of treatment on dietary intake, fasting breath hydrogen, AUC and iAUC of postprandial data, gastrointestinal symptom mean daily ratings, HOMA and postprandial insulin sensitivity estimates were investigated by one way repeated measures ANOVA with post-hoc Bonferonni or Friedman test with post-hoc Wilcoxon signed ranks test as appropriate. Postprandial data was also analysed by two-way (treatment x time) repeated measures ANOVA. Data are presented as mean \pm SD unless otherwise stated and differences were considered significant at $p \leq 0.05$ except post-hoc Wilcoxon, where significance was set at $p \leq 0.0167$ ($=0.05/3$).

The sample size was based on the chronic crossover study of Vogt et al. in which a significant increase in serum propionate was found with L-rhamnose supplementation in 11 participants (Vogt

et al., 2004a). As we wanted to investigate if a rise in serum propionate would drive effects on appetite, we aimed to recruit at least 11 participants to investigate these mechanisms. A power analysis was performed retrospectively in which we found 13 participants would have given an 80% power of detecting a difference in actual food intake of 749 kJ, with a measured SD of the response to L-rhamnose treatment of 845 kJ. As the actual net effect of the L-rhamnose ingestion was a reduced food intake of only 150 kJ, with 5/13 participants eating more following the L-rhamnose than following control, 250 participants would have been required at 80% power in order to achieve statistical significance, which far exceeds other studies of this nature by several fold. The lack of statistical effect is therefore likely to be due to a lack of biological effect rather than simply a power issue.

3. Results

Thirteen participants aged 19–32 y with BMI and DEBQ restraint score ranging from 19.5 to 24.7 kg/m² and 1.1 to 3.6 respectively completed the study. Of the eight female participants, seven were using birth control medication (Table 1). Results are presented for the run-in period (days 1–6) and for the study day (day 7).

3.1. Quantitative appetite assessment

No differences between treatments were found for mean daily energy and macronutrient intake during the days 3–6 of the run-in period, or for 24-h dietary intake on the study day (Table 2). Mean *ad libitum* EI of the homogenous pasta meal served at 420 min on the study day did not differ between control, inulin or L-Rha (4202 ± 1666, 4089 ± 1680 and 4053 ± 1538 kJ respectively).

3.2. Qualitative appetite assessment

No treatment or treatment × time effects were found for postprandial VAS appetite ratings on the study day for hunger (Fig. 1A), fullness, prospective consumption or desire to eat a snack, and AUC did not differ between treatments. A treatment × time interaction was found during the morning (0–180 min) for meal desire (F(12,144) = 2.36, *P* = 0.008) (Fig. 1B) and during the afternoon (180–420 min) for the desire to eat sweet (F(16,192) = 1.78, *P* = 0.036), but no difference between treatments was found by post-hoc analysis.

3.3. Breath hydrogen concentrations

Significant treatment effects were found for fasting and postprandial AUC breath hydrogen concentrations ($\chi^2(2) = 14.3$, *P* = 0.001 and $\chi^2(2) = 15.9$, *P* < 0.001 respectively) (Fig. 1C). Fasting concentrations were significantly higher following inulin than L-

Rha (T = 89, *r* = −0.49, *P* = 0.002) and control (T = 82, *r* = −0.41, *P* = 0.011), and the AUC was significantly higher for inulin (T = 91, *r* = −0.51, *P* = 0.001) and L-Rha (T = 83, *r* = −0.42, *P* = 0.009) than control.

3.4. Postprandial metabolites

For postprandial insulin concentrations (Fig. 2A) treatment effects approaching significance following breakfast (F(2,24) = 3.38, *P* = 0.051) and significant treatment × time effects following breakfast (F(16,192) = 1.88, *P* = 0.024), lunch (F(18,216) = 1.81, *P* = 0.026) and during the entire study day (F(34, 408) = 1.71, *P* = 0.009) were found. The iAUC following breakfast and during the entire study day were significantly lower for L-Rha than control (T = 9, *r* = −0.41, *P* = 0.011 and T = 13, *r* = −0.36, *P* = 0.023 respectively) (Fig. 2B). No treatment or treatment × time interactions for postprandial glucose or triglycerides and no treatment effects for postprandial NEFA concentrations were found, although there was a significant treatment × time interaction (F(16,192) = 1.72, *P* = 0.046) following lunch for NEFA.

3.5. Serum SCFA

No treatment or treatment × time interactions or differences between AUC or iAUC were found postprandially and no differences between fasting concentrations were found between treatments for propionate, acetate or butyrate (Fig. 3).

3.6. Insulin sensitivity

Neither fasting insulin sensitivity, cell function and insulin resistance as estimated by HOMA nor postprandial insulin sensitivity estimated using the minimal model method were found to differ between treatments (data not shown).

3.7. Gastrointestinal symptoms

During run-in, the mean daily flatulence ratings during days 4–6 (when participants were consuming the target dose of supplement) were significantly influenced by treatment ($\chi^2(2) = 8.6$, *P* = 0.014). Scores were significantly higher during inulin treatment than control (T = 36, *r* = −0.40, *P* = 0.012) and L-Rha (T = 6, *r* = −0.35, *P* = 0.028), with mean scores of 2.2 ± 0.9, 1.4 ± 0.6 and 1.5 ± 0.5 respectively. On the study day (day 7), the AUC for the urge to defecate was significantly higher during L-Rha treatment than control (T = 84, *r* = −0.43, *P* = 0.007) (data not shown). None of the other gastrointestinal symptoms was significantly influenced by treatment during run-in or on the study day.

Table 1

Baseline characteristics of participants at screening. Data shown as mean ± SD and n (%).

	Overall (n = 13)	Male (n = 5)	Female (n = 8)
Age/y	23 ± 4	23 ± 3	23 ± 4
BMI/kgm ⁻²	22.1 ± 1.6	23.0 ± 1.5	21.5 ± 1.5
Waist circumference/cm	75.5 ± 6.9	82.9 ± 3.1	70.5 ± 2.3
Body fat/%	21.9 ± 6.5	15.5 ± 3.4	26.0 ± 4.1
Systolic BP/mmHg	115 ± 9	120 ± 7	110 ± 8
Diastolic BP/mmHg	68 ± 8	67 ± 6	69 ± 9
Fasting blood glucose/mmol/L	4.3 ± 0.5	4.4 ± 0.5	4.2 ± 0.4
DEBQ Restraint Score	2.1 ± 0.8	2.2 ± 0.9	2.1 ± 0.7
Using birth control/n (%)			7 (88%) ^a

Abbreviations: DEBQ, Dutch Eating Behaviour Questionnaire Restraint Score, BMI, body mass index, BP, blood pressure.

^a 5 using combined pill, 1 using contraceptive implant, 1 using progesterone-only pill.

Table 2
(A) Mean daily intake during last 4-d of run-in period, and (B) 24-h intake on study day, including all provided foods during study and additional intake for the remainder of the day in 13 healthy men and women during supplementation with inulin, L-Rhamnose (L-Rha) or control. No differences were found between treatments. Analyses carried out by one-way within participants repeated measures ANOVA or Friedman's as appropriate. Data shown as mean \pm SD.

	(A) daily intake during run-in			(B) 24-h intake on study day		
	Control	Inulin	L-Rha	Control	Inulin	L-Rha
Energy/kJ	9078 \pm 1857	8730 \pm 2232	8691 \pm 2131	12368 \pm 3128	11667 \pm 1930	12383 \pm 2656
Energy excluding alcohol/kJ	8822 \pm 1901	8266 \pm 1985	8138 \pm 1734	11964 \pm 2162	11210 \pm 1765	11500 \pm 1245
Fat/% E	33.5 \pm 5.9	31.9 \pm 5.6	33.0 \pm 4.7	31.6 \pm 3.3	30.3 \pm 2.9	32.9 \pm 2.6
Protein/%E	14.7 \pm 3.1	14.1 \pm 2.5	14.3 \pm 2.4	13.9 \pm 1.8	13.8 \pm 1.3	13.6 \pm 1.5
Carbohydrate/%E	49.0 \pm 5.2	49.3 \pm 7.2	47.4 \pm 7.8	52.4 \pm 3.5	53.2 \pm 3.6	51.1 \pm 3.9
Alcohol/%E	2.9 \pm 4.0	4.7 \pm 5.8	5.3 \pm 7.9	2.0 \pm 6.2	2.8 \pm 5.9	2.3 \pm 3.7

3.8. Taste ratings

Mean daily ratings of the taste of the jellies supplied during the run-in period did not differ between treatments. On the study day, treatment significantly influenced the rated taste of the mouse at breakfast ($F(2,24) = 5.49, P = 0.011$), with the lowest VAS score for L-Rha, followed by inulin and the highest for control ($58 \pm 21, 73 \pm 20$ and 75 ± 21 mm respectively), but not at lunch.

4. Discussion

Our findings suggest supplementation with 25.5 g/d L-Rha or 22.4 g/d inulin HP significantly influence postprandial plasma insulin ($P = 0.009$) and plasma NEFA ($P = 0.046$, following lunch) responses. The lowest response for both was with L-Rha treatment, previously reported to enhance serum propionate concentrations (Vogt et al., 2004a, b), which was to a significant level for plasma insulin in comparison to control. This was accompanied by significant increases in postprandial breath hydrogen concentrations ($P < 0.001$) following L-Rha and inulin ingestion in comparison to control, indicating colonic fermentation occurred, although serum SCFA concentrations were not increased alongside this. However quantitative and subjective appetite measures were not altered with supplementation, except desire to eat ($P = 0.008$) during the morning, suggestive of a lack of effect of these non-digestible carbohydrate supplements on appetite.

The observed suppression of postprandial plasma insulin following L-Rha may have arisen via propionate-mediated activation of FFAR2 and/or FFAR3 in colonic mucosa initiating ileal brake mechanisms via PYY and GLP-1 production (Karaki et al., 2006; Karaki et al., 2008; Tazoe et al., 2009). However, unlike previous reports (Vogt et al., 2004a, b) we found no impact on serum propionate concentrations following L-Rha ingestion. Previous studies found L-Rha supplementation did not alter postprandial insulin or NEFA responses (Vogt et al., 2006), in contrast with the present study, nor postprandial plasma glucose and triglyceride responses (Vogt et al., 2006; Vogt et al., 2004b), in common with the present study. A limitation of previous investigations is that glucose was used as control, which could confound interpretation, and blood samples were collected only hourly, therefore potentially missing postprandial effects of these metabolites, which change rapidly following a meal. By contrast the present study examined effects of L-Rha on postprandial metabolites with regular blood sampling and an appropriate control, explaining why our results reveal novel findings in contrast to those before. The effects we observed did not appear to be related to insulin sensitivity, as we found no influence of L-Rha or inulin on estimates of fasting and postprandial insulin sensitivity.

To our knowledge this is the first study to investigate effects of L-Rha on appetite. In contrast to the lack of effect on appetite in the present study, colonic delivery of propionate while tethered to

inulin significantly increased postprandial PYY and GLP-1 responses and reduced EI acutely and weight gain chronically (Chambers et al., 2015). Possibly greater colonic concentrations of propionate were achieved with this novel non-digestible carbohydrate than via L-Rha, arguably a natural source of propionate, in the present study. This is supported by our finding that serum propionate concentrations were not altered by treatment. Further, in contrast to the present study, the novel non-digestible carbohydrate did not significantly alter postprandial insulin concentrations (Chambers et al., 2015). Possibly the effects on insulin following L-Rha supplementation may not be potentiated by the colonic generation of propionate, but by some other, as yet unknown, mechanism.

Relatively few previous investigations have reported on effects on appetite of long-chain inulin (Archer et al., 2004; Karalus et al., 2012; Tarini & Wolever, 2010) like that used in the present study, with the majority of studies reporting on supplementation with short-chain inulin-type fructans (for example Hess et al., 2011; Parnell & Reimer, 2009; Pedersen et al., 2013; Peters et al., 2009; Verhoef et al., 2011). Long-chain inulin has the benefit of being associated with less adverse gastrointestinal symptoms than the short-chain counterpart (Bonnema, Kolberg, Thomas, & Slavin, 2010; Bruhwiler, Carreer, Demanet, & Jacobs, 2009), providing a rationale to investigate effects on long-chain inulin. Indeed in the present study we found that gastrointestinal symptoms were not adversely affected by long-chain inulin supplementation, except significantly higher flatulence scores during run-in, and even then the mean flatulence symptom score with inulin treatment of 2.2 ± 0.9 (scored on a 9-point Likert Scale) remained relatively low.

We did not find any significant effects of supplementation with long-chain inulin on qualitative or quantitative appetite measures, on postprandial metabolites or on serum SCFA concentrations. The lack of effect on postprandial serum SCFA in conjunction with a higher breath hydrogen response has been previously reported following consumption of long-chain inulin (Fernandes et al., 2011). In common with our findings, a previous trial in 22 unrestrained females found acute ingestion of 10 g long-chain inulin within a chocolate crisp bar did not alter qualitative or quantitative appetite measures in comparison to a control bar (Karus et al., 2012). By contrast 24 g long-chain inulin used as a fat-replacer in sausage patties significantly reduced 24-h EI in comparison to a full fat patty in an acute meal challenge in healthy participants ($n = 33$), although rated satiety was not altered, and *ad libitum* EI was not investigated (Archer et al., 2004). However, the control had a higher fat and therefore energy content than the inulin preload making interpretation difficult (Archer et al., 2004). In our study the control was energy and macronutrient matched to the investigative products. Higher plasma GLP-1 concentrations at 30 min and reduced ghrelin concentrations at 270 and 360 min were reported following an acute meal challenge in 12 healthy participants with 24 g inulin plus 56 g high-fructose corn syrup in comparison to 56 g high-

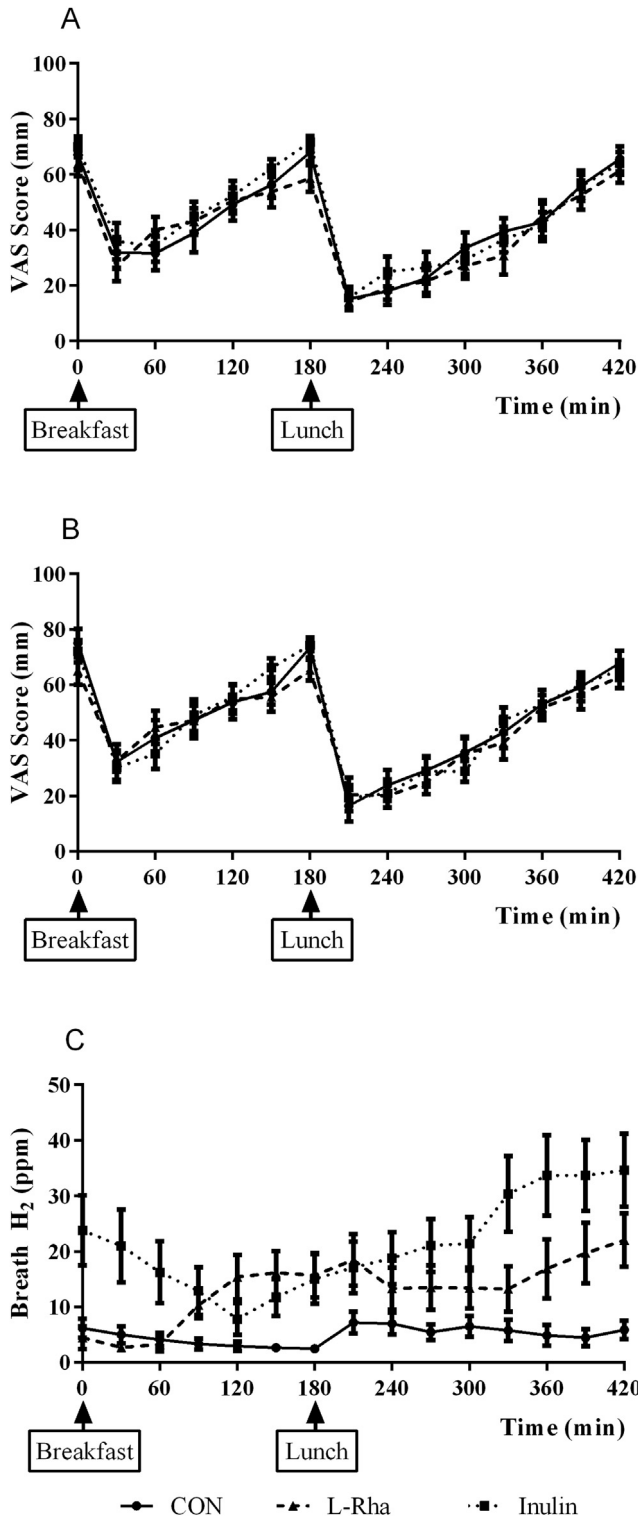


Fig. 1. Postprandial appetite ratings for (A) hunger, (B) desire to eat a meal, and (C) postprandial breath hydrogen in 13 healthy men and women after consuming a mixed breakfast and lunch that included inulin (■), l-rhamnose (▲) or control (●) following a 6-day run-in period. Values are mean with error bars representing the SEM.

fructose corn syrup and 80 g high-fructose corn syrup mixed into a drink (Tarini & Wolever, 2010). However as effects on GLP-1 were seen so early on postprandially this is suggestive the observation is not linked to colonic fermentation. Overall the evidence does not

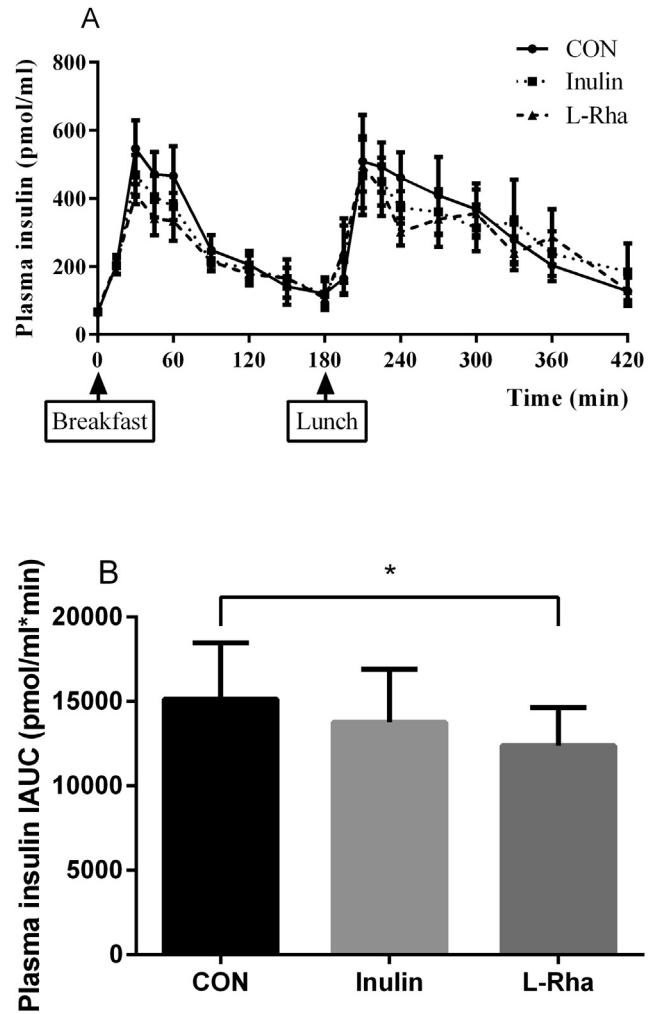


Fig. 2. Postprandial plasma insulin (A) timecourse curve and (B) incremental area under curve in 13 healthy men and women after consuming a mixed breakfast and lunch that included inulin (■), l-rhamnose (▲) or control (●) following a 6-day run-in period. Values are mean with error bars representing the SEM.

strongly support a role for long-chain inulin in influencing appetite, although studies are limited by participant numbers.

The majority of previous studies investigating metabolic effects of long-chain inulin have investigated fasting rather than postprandial effects, therefore there is a paucity of comparative studies. In an acute meal challenge, glucose and insulin responses did not differ significantly following supplementation with 24 g inulin plus 56g high-fructose corn syrup mixed into a drink in comparison to 56 g and 80 g high-fructose corn syrup in healthy participants (n = 12) (Tarini & Wolever, 2010), agreeing with findings from the present study. Similarly there was no difference in postprandial glucose and insulin in response to an oral glucose tolerance test in men classified at higher risk of cardiovascular disease (n = 10) following consumption of 15 g inulin per day within bread rolls for 4 weeks in comparison to nutrient-matched control bread rolls (Tripkovic, Muirhead, Hart, Frost, & Lodge, 2015).

The present study had a number of limitations which need to be acknowledged when interpreting findings. Ideally the study would have been double blinded, however as there was only a single investigator (JD) to conduct the study this was not possible. The study included relatively few participants, however effects on food intake were so negligible retrospective power analysis suggested

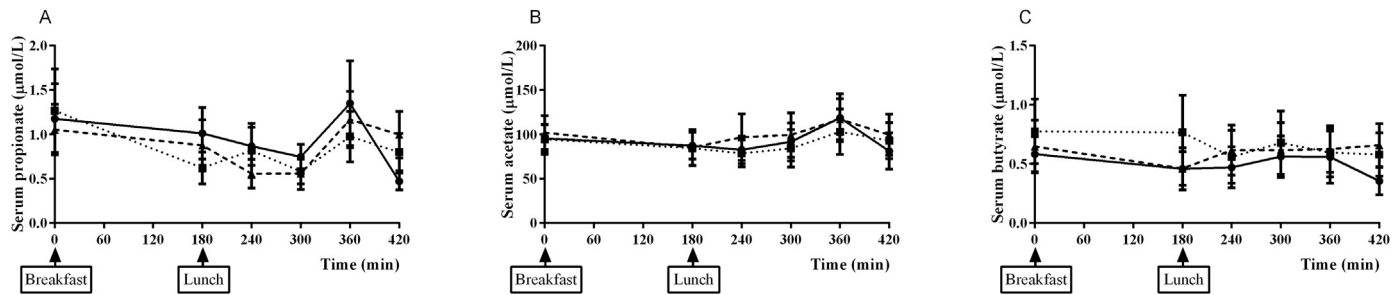


Fig. 3. Postprandial serum (A) propionate, (B) acetate and (C) butyrate in 13 healthy men and women after consuming a mixed breakfast and lunch that included inulin (■), L-rhamnose (▲) or control (●) following a 6-day run-in period. Values are mean with error bars representing the SEM.

250 participants would be required for statistical significance, which is far greater than in other studies of this nature. Thus the lack of effect is likely due to a lack of biological effect rather than simply a power issue. We chose to include female participants in order to be more representative of the general population. However whilst every effort was made to control for hormonal fluctuations, this will likely have added some variability. Participants were informed of the broad purpose of the study, which may have impacted behavior, however every effort was made to maintain a uniform protocol for each condition. We did not assess if participants were aware of the study hypotheses or if they were able to accurately report any differences in the protocol according to the condition they were randomised to. As water was supplied *ad libitum* to be more reflective of free-living conditions, this may have impacted on appetite, an effect we could not assess as water intake was not measured.

In conclusion, the present investigation found neither inulin HP nor L-Rha influenced appetite, and inulin HP did not influence postprandial metabolic responses. However L-Rha appeared to inhibit postprandial insulin secretion and also NEFA, possibly via propionate-mediated colonic FFAR2 and/or FFAR3 actions, although serum propionate was not significantly altered and insulin was not inhibited following ingestion of propionate tethered to inulin. This suggests that the mechanism underlying our findings may not be mediated by propionate as originally hypothesized, which warrants further investigation.

Conflict of interest disclosure

None to declare.

Author contributions

JD, MDR and GSF designed the research. JD conducted the research, analysed the data and wrote the paper. MDR and GSF refined the paper. JS and AC analysed the serum samples for SCFA concentrations and refined the paper. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to all the volunteers who participated in this research and also to Dr Shelagh Hampton, John Wright and Nicola Muirhead for medical assistance and cannulation expertise. JD was supported by an educational fellowship from Premier Foods (P00417) and inulin was supplied by DKSH Great Britain Limited.

References

Archer, B. J., Johnson, S. K., Devereux, H. M., & Baxter, A. L. (2004). Effect of fat

- replacement by inulin or lupin-kernel fibre on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. *British Journal of Nutrition*, *91*, 591–599.
- Asarian, L., & Geary, N. (2013). Sex differences in the physiology of eating. *American Journal of Physiology Regulatory Integrative and Comparative Physiology*, *305*, R1215–R1267.
- Bodinham, C. L., Frost, G. S., & Robertson, M. D. (2010). Acute ingestion of resistant starch reduces food intake in healthy adults. *British Journal of Nutrition*, *103*, 917–922.
- Bodinham, C. L., Smith, L., Thomas, E. L., Bell, J. D., Swann, J. R., Costabile, A., et al. (2014). Efficacy of increased resistant starch consumption in human type 2 diabetes. *Endocrine Connections*, *3*, 75–84.
- Bonnema, A. L., Kolberg, L. W., Thomas, W., & Slavin, J. L. (2010). Gastrointestinal tolerance of chicory inulin products. *Journal of American Dietetic Association*, *110*, 865–868.
- Brown, A. J., Goldsworthy, S. M., Barnes, A. A., Eilert, M. M., Tcheang, L., Daniels, D., et al. (2003). The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *Journal of Biological Chemistry*, *278*, 11312–11319.
- Bruhwyler, J., Carreer, F., Demanet, E., & Jacobs, H. (2009). Digestive tolerance of inulin-type fructans: a double-blind, placebo-controlled, cross-over, dose-ranging, randomized study in healthy volunteers. *International Journal of Food Sciences and Nutrition*, *60*, 165–175.
- Burton-Freeman, B. (2000). Dietary fiber and energy regulation. *Journal of Nutrition*, *130*, 272S–275S.
- Caumo, A., Bergman, R. N., & Cobelli, C. (2000). Insulin sensitivity from meal tolerance tests in normal subjects: a minimal model index. *Journal of Medical Endocrinology and Metabolism*, *85*, 4396–4402.
- Chambers, E. S., Viardot, A., Psichas, A., Morrison, D. J., Murphy, K. G., Zaccaghese, S. E., et al. (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut*, *64*, 1744–1754.
- Cherbut, C., Ferrier, L., Rozé, C., Anini, Y., Blottière, H., Lecannu, G., et al. (1998). Short-chain fatty acids modify colonic motility through nerves and polypeptide YY release in the rat. *American Journal of Physiology*, *275*, G1415–G1422.
- Covington, D. K., Briscoe, C. A., Brown, A. J., & Jayawickreme, C. K. (2006). The G-protein-coupled receptor 40 family (GPR40–GPR43) and its role in nutrient sensing. *Biochemical Society Transactions*, *34*, 770–773.
- Cuche, G., Cuber, J. C., & Malbert, C. H. (2000). Ileal short-chain fatty acids inhibit gastric motility by a humoral pathway. *American Journal of Physiology Gastrointestinal and Liver Physiology*, *279*, G925–G930.
- Darzi, J., Frost, G. S., & Robertson, M. D. (2011). Do SCFA have a role in appetite regulation? *Proceedings of the Nutrition Society*, *70*, 119–128.
- Darzi, J., Frost, G. S., & Robertson, M. D. (2012). Effects of a novel propionate-rich sourdough bread on appetite and food intake. *European Journal of Clinical Nutrition*, *66*, 789–794.
- Du, H., van der A., D. L., Boshuizen, H. C., Forouhi, N. G., Wareham, N. J., Halkjaer, J., et al. (2010). Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. *American Journal of Clinical Nutrition*, *91*, 329–336.
- Fernandes, J., Rao, A. V., & Wolever, T. M. (2000). Different substrates and methane producing status affect short-chain fatty acid profiles produced by in vitro fermentation of human feces. *Journal of Nutrition*, *130*, 1932–1936.
- Fernandes, J., Vogt, J., & Wolever, T. M. (2011). Inulin increases short-term markers for colonic fermentation similarly in healthy and hyperinsulinaemic humans. *European Journal of Clinical Nutrition*, *65*, 1279–1286.
- Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders*, *24*, 38–48.
- Hess, J. R., Birkett, A. M., Thomas, W., & Slavin, J. L. (2011). Effects of short-chain fructooligosaccharides on satiety responses in healthy men and women. *Appetite*, *56*, 128–134.
- Howarth, N. C., Huang, T. T., Roberts, S. B., & McCrory, M. A. (2005). Dietary fiber and fat are associated with excess weight in young and middle-aged US adults.

- Journal of American Dietetic Association*, 105, 1365–1372.
- Hussain, S. S., & Bloom, S. R. (2013). The regulation of food intake by the gut-brain axis: implications for obesity. *International Journal of Obesity*, 37, 625–633.
- Karaki, S., Mitsui, R., Hayashi, H., Kato, I., Sugiya, H., Iwanaga, T., et al. (2006). Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Research*, 324, 353–360.
- Karaki, S., Tazoe, H., Hayashi, H., Kashiwabara, H., Tooyama, K., Suzuki, Y., et al. (2008). Expression of short-chain fatty acid receptor, GPR43, in the human colon. *Journal of Molecular Histology*, 39, 135–142.
- Karalus, M., Clark, M., Greaves, K. A., Thomas, W., Vickers, Z., Kuyama, M., et al. (2012). Fermentable fibers do not affect satiety or food intake by women who do not practice restrained eating. *Journal of Academy of Nutrition and Dietetics*, 112, 1356–1362.
- Kristensen, M., & Jensen, M. G. (2011). Dietary fibres in the regulation of appetite and food intake. Importance of viscosity. *Appetite*, 56, 65–70.
- Le Poul, E., Loison, C., Struyf, S., Springael, J. Y., Lannoy, V., Decobecq, M. E., et al. (2003). Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *Journal of Biological Chemistry*, 278, 25481–15489.
- Lean, M. E., & Malkova, D. (2015). Altered gut and adipose tissue hormones in overweight and obese individuals: cause or consequence? *International Journal of Obesity*. <http://dx.doi.org/10.1038/ijo.2015.220> [Epub ahead of print].
- Levy, J. C., Matthews, D. R., & Hermans, M. P. (1998). Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care*, 21, 2191–2192.
- Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28, 412–419.
- Parnell, J. A., & Reimer, R. A. (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *American Journal of Clinical Nutrition*, 89, 1751–1759.
- Pedersen, C., Lefevre, S., Peters, V., Patterson, M., Ghatei, M. A., Morgan, L. M., et al. (2013). Gut hormone release and appetite regulation in healthy non-obese participants following oligofructose intake. *Appetite*, 66, 44–53.
- Peters, H. P., Boers, H. M., Haddeman, E., Melnikov, S. M., & Qvyyt, F. (2009). No effect of added beta-glucan or of fructooligosaccharide on appetite or energy intake. *American Journal of Clinical Nutrition*, 89, 58–63.
- Psichas, A., Sleeth, M. L., Murphy, K. G., Brooks, L., Bewick, G. A., Hanyaloglu, A. C., et al. (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *International Journal of Obesity*, 39, 424–429.
- Robertson, M. D., Bickerton, A. S., Dennis, A. L., Vidal, H., & Frayn, K. N. (2005). Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. *American Journal of Clinical Nutrition*, 82, 559–567.
- Tarini, J., & Wolever, T. M. (2010). The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Applied Physiology, Nutrition, and Metabolism*, 35, 9–16.
- Tazoe, H., Otomo, Y., Karaki, S., Kato, I., Fukami, Y., Terasaki, M., et al. (2009). Expression of short-chain fatty acid receptor GPR41 in the human colon. *Biomedical Research*, 30, 149–156.
- Tripkovic, L., Muirhead, N. C., Hart, K. H., Frost, G. S., & Lodge, J. K. (2015). The effects of a diet rich in inulin or wheat fibre on markers of cardiovascular disease in overweight male subjects. *Journal of Human Nutrition and Dietetics*, 28, 476–485. <http://dx.doi.org/10.1111/jhn.12251>.
- Van Strien, T., Frijters, J. E. R., Bergers, G. P. A., & Defares, P. B. (1986). The Dutch eating behavior questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. *International Journal of Eating Disorders*, 5, 295–315.
- Verhoef, S. P., Meyer, D., & Westerterp, K. R. (2011). Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. *British Journal of Nutrition*, 106, 1757–1762.
- Vogt, J. A., Ishii-Schrade, K. B., Pencharz, P. B., Jones, P. J., & Wolever, T. M. (2006). L-rhamnose and lactulose decrease serum triacylglycerols and their rates of synthesis, but do not affect serum cholesterol concentrations in men. *Journal of Nutrition*, 136, 2160–2166.
- Vogt, J., Ishii-Schrade, K. B., Pencharz, P. B., & Wolever, M. S. (2004a). L-Rhamnose increases serum propionate after long-term supplementation, but lactulose does not raise serum acetate. *American Journal of Clinical Nutrition*, 80, 1254–1261.
- Vogt, J. A., Pencharz, P. B., & Wolever, M. S. (2004b). L-Rhamnose increases serum propionate in humans. *American Journal of Clinical Nutrition*, 80, 89–94.
- Wallace, T. M., Levy, J. C., & Matthews, D. R. (2004). Use and abuse of HOMA modeling. *Diabetes Care*, 27, 1487–1495.
- Wanders, A. J., van den Borne, J. J., de Graaf, C., Hulshof, T., Jonathan, M. C., Kristensen, M., et al. (2011). Effects of dietary fibre on subjective appetite, energy intake and body weight: a systematic review of randomized controlled trials. *Obesity Reviews*, 12, 724–729.
- Weststrate, J. A., & van Amelsvoort, J. M. (1993). Effects of the amylose content of breakfast and lunch on postprandial variables in male volunteers. *American Journal of Clinical Nutrition*, 58, 180–186.
- Xiong, Y., Miyamoto, N., Shibata, K., Valasek, M. A., Motoike, T., Kedzierski, R. M., et al. (2004). Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. *Proceedings of National Academy of Sciences USA*, 101, 1045–1050.