European Journal of Clinical Nutrition (1999) 53, 726–733

http://www.stockton-press.co.uk/ejcn

Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers

F Brighenti[1]*, MC Casiraghi [1], E Canzi[2] and A Ferrari[2]

[1]*Department of Food Science, Technology and Microbiology, Division of Human Nutrition and;* [2]*Division of Microbiology, University of Milan, Italy*

Objective: To investigate the effect of a breakfast cereal containing inulin on blood lipids and colonic ecosystem in normolipidemic young men.
Setting: Department of Food Science and Microbiology,University of Milan, Italy.
Subjects: Twelve healthy male volunteers, age 23.3 ± 0.5 y, body mass index (BMI) 25.7 ± 1.2 kg/m^2 (mean $\pm$ s.e.m.).
Interventions: Subjects consumed daily, for three periods of four weeks, 50 g of a rice-based ready-to-eat cereal (placebo) and the same cereal containing 18% inulin (test) in substitution of their habitual breakfast, then returned to the habitual diet (wash-out). They followed no other dietary restrictions.
Results: No changes in body weight, dietary habits, faecal and bile acid output, faecal short-chain fatty acid (SCFA) and faecal pH, were observed at the end of each period, whereas plasma total cholesterol and triacylglycerols significantly decreased at the end of test period by 7.9 ± 5.4 ($P < 0.05$) and 21.2 ± 7.8% ($P < 0.005$) respectively. Meal glucose tolerance test (MTT) resulted in the same incremental area under the curve for both cereals (IAUC test 124 ± 35; placebo 118 ± 33 mmol·min/l, ns). Inulin markedly enhanced breath H_2 excretion (IAUC test 280 ± 40; placebo 78 ± 26 ppm·h, $P < 0.005$), as well as faecal concentration of L-lactate. Total facultative anaerobes significantly decreased after test, and bifidobacteria increased after correction for total anaerobes ($P < 0.05$). Changes in blood lipids were negatively correlated with bifidobacteria counts and positively with secondary bile acid excretion ($P < 0.05$).
Conclusions: Inulin seems to have a lipid lowering potential in normolipidemic men possibly mediated by mechanisms related to colonic fermentation.
Sponsorship: National Research Council of Italy, grant number 95.00773.PF41.
Descriptors: inulin; plasma lipids; fermentation; colonic microflora; bile acids

Introduction

Inulin (degree of polymerization DP > 20) and fructo-oligosaccharides (FOS; DP < 20) are naturally-occurring polyfructans found in different vegetables as storage carbohydrates (Van Loo *et al*, 1995). Intake of polyfructans in the diet has been estimated to vary from 3 to 12 g/day in Western countries (Van Loo *et al*, 1995). Purified inulin and FOS have been recently introduced on the market as food ingredients since they display gelling and thickening properties that do not interfere with the texture or the taste of foods and, being resistant to hydrolysis by mammalian enzymes in the gut, can act as hypocaloric replacers for sugar and fat. Once in the colon, inulin and FOS are completely fermented by the colonic microflora, with production of gases and monocarboxilic acids, mainly short-chain fatty acids (SCFA) and L-lactic acid (Roberfroid, 1993). Thanks to their ability to selectively stimulate the growth of bifidobacteria at the expense of bacteroides, clostridia, or coliforms, polyfructans are considered prebiotic factors (reviewed by Roberfroid *et al*, 1998). In addition to their bifidogenic properties, inulin and FOS have been claimed to influence lipid metabolism. However, these claims mainly originate from animal studies. Administration of polyfructan-containing diets to male rats consistently resulted in a decrease in plasma triacylglycerols, phospholipids and cholesterol (Fiordaliso *et al*, 1995; Delzenne *et al*, 1993; Vanhoof & DeSchrijver, 1995; Kok *et al*, 1996). On the other hand, indications of a lipid-lowering effect in humans are still limited and conflicting. In non-insulin dependent diabetes (NIDDM) diabetics, consumption of 8 g/d of FOS for two weeks resulted in a decrease of 8% and 10% in total and low-density lipoprotein (LDL) cholesterol respectively (Yamashita *et al*, 1984). In hypercholesterolemic patients, supplementation of foods (chocolate, spreads and sweeteners) containing 18 g/d of inulin to a National Cholesterol Education Program Step 1 diet for six weeks did not change total and LDL-cholesterol levels, but significantly prevented the increase of blood lipids observed during the control period when the same foods without inulin were given (Davidson *et al*, 1998). In contrast, no effect on plasma lipid was observed in young healthy women after consumption of

*Correspondence: Furio Brighenti, Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche , Università degli Studi di Milano, 2 Via G.Celoria, 20133 Milan, Italy.
Email furio.brighenti@unimi.it
Guarantors: Furio Brighenti and Enrica Canzi.
Received 20 November 1998; revised 17 March, 1999; accepted 2 April 1999

14 g/d of inulin incorporated into a fat spread for 4 weeks (Pedersen *et al*, 1997). Therefore, the potential of polyfructans as functional ingredients able to reduce blood lipid levels in the general population, as well as the mechanisms of action, remain to be elucidated.

The present study was performed to investigate the effect on the lipid metabolism of young healthy men during a four-week ingestion of a ready-to-eat (RTE) breakfast cereal containing inulin. This effect was also studied in relation to changes in the colonic microflora, metabolites of bacterial fermentation and bile acid excretion.

Subjects and methods

Young, healthy male volunteers were recruited for the study by posting adverts in the students' room of the Faculty of Agriculture of the University of Milan. Candidates were selected to meet the following inclusion criteria: normal body weight or moderately overweight (body mass index (BMI) > 20 and $< 30\,kg/m^2$); normal dietary habits, excluding vegetarianism or therapeutic diets; low to normal physical exercise (< 60 min exercise three times per week); no drug therapy, including any kind of food supplements; normal or moderately raised blood lipids (fasting triacylglycerols < 2.0 mmol/l, fasting plasma cholesterol < 5.5 mmol/l). Twelve male volunteers, (age 23.3 ± 0.5 y, BMI $25.7 \pm 1.2\,kg/m^2$ (mean $\pm$ sem)) were selected and studied for a total of twelve weeks after having signed an informed consent form. During the first four weeks they were asked to consume daily 50 g of a RTE rice cereal plus 200 ml of 2% fat milk (placebo), and during the second four weeks 50 g of a RTE rice cereal containing inulin (test) and the same amount of milk in partial or total substitution of their habitual breakfast (basal). Subjects were not aware of the differences between the cereals, which were indistinguishable regarding their organoleptic qualities. During the last four weeks they returned to their habitual diet (wash-out). They followed no other dietary restrictions. During the entire study volunteers were asked to maintain an identical pattern of physical activity, not to change drastically their diet and not to take antibiotics and other drugs or dietary supplements. Volunteers were asked to came to the laboratory four times during the study (*i.e.* baseline, end of placebo, end of test, and end of wash-out periods) in the morning after an overnight fast. At baseline and at the end of each period, 20 ml of blood were taken by venipuncture for lipid analysis. Fasting total cholesterol, high-density lipoprotein cholesterol (HDL) and total triacylglycerols (TAG) were determined on serum samples stored at $-20^{\circ}C$ using a dedicated automatic analyser (Kone Specific Selective Chemistry Analyser, KONE Instruments S.A, Evry, France). LDL cholesterol was derived by using the Friedewald formula.

Volunteers then underwent a physical examination and answered a semiquantitative food frequency questionnaire to estimate food habits during the previous month. The questionnaire, designed and validated to evaluate food intake from typical Italian food patterns (Fidanza *et al*, 1995), was administered by the same trained dietician. The protocol of the study followed the rules of the University of Milan Ethical Committee.

Cereals

The inulin used in this study (Fibruline Instant, Cosucra, Momalle, Belgium) was a mixture of linear β $(2\rightarrow1)$ fructose polymers, with an average DP of 7 fructosyl- and 1 glucosyl residues, purified from chicory root. According to the supplier, at least 30% of the product had a degree of polymerization of 30 sugar units or more, and therefore should be considered as a mixture of inulin and FOS. Inulin was incorporated at a level of 18% (dry weight) in the test cereal, prepared by puffing a dough made with rice flour, salt, sucrose, maltodextrins, and water in a single-screw extrusion pilot plant. The placebo cereal contained the same ingredients apart from inulin.

Intestinal habits and faecal composition

Intestinal habits were estimated using a self-administered questionnaire, filled in by subjects at the beginning of the study and during the last 3 d (Friday, Saturday and Sunday) of the third week of the placebo and test periods. After each defecation subjects marked the weight of faeces, measured on a portable electronic scale provided for them, and answered questions about the ease of defecation and faeces consistency (linear scale) and about abdominal pain and flatulence (yes or no).

The pH was measured directly on the sample with a pH electrode (mod. pHM 83 Autocal, Biorad). Faecal short chain fatty acids (SCFAs) were measured on the supernatant obtained by centrifugation of faeces diluted 1:1 v/v with saline, using an HPLC method after vacuum distillation (Scheppach *et al*, 1987). Faecal L-lactic acid was measured after further dilution with saline by means of a dedicated analyser (Yellow Spring Inst. model 2300, Yellow Spring, Ohio).

Faecal microbial analysis

At baseline and on the fourth day of the last week of each period, a sample of about 40 g of fresh faeces was taken from the first stool passed in the morning, and delivered to the laboratory within an hour from defecation.

The faecal specimen was immediately processed under strict anaerobic conditions in an anaerobic cabinet (Forma Scientific, Marietta, Ohio, USA) under an atmosphere of N_2:H_2:CO_2/85:10:5 v/v. A sub-sample of about 5 g was suspended in 50 ml of prereduced dilution blank (Holdeman & Moore, 1973) and homogenized; serial tenfold dilutions to 10^{10} were subsequently performed in the same dilution blank. Microbial quantitative and qualitative analyses were performed using selective growth media, by examining the following microbial groups: total anaerobes on blood agar (Holdeman & Moore, 1973), total facultative anaerobes on Difco Tryptic Soy agar, bifidobacteria on NPNL-agar (Teraguchi *et al*, 1978), *Bacteroidaceae* on kanamycin–vancomycin blood agar medium (Finegold *et al*, 1971), clostridia on sulfite–polymixin–milk agar medium (Mevissen-Verhage *et al*, 1982), and coliforms on Difco levine–eosine–methylene–blue agar. All media used for cultivation of anaerobe micro-organisms were prereduced in the anaerobic cabinet for 48 h before use. Micro-organisms were classified on the basis of morphological, cultural and physiological properties according to V.P.I. Anaerobe Laboratory Manual (Holdeman & Moore, 1973). Microbial counts were reported to the dry weight of the faecal inoculum, determined by placing 5-ml aliquots of the initial suspension in weighing bottles and drying in an oven at 100°C until constant weight.

Bile acids analysis
Stools passed from 20:00 of the fourth to 20:00 of the fifth day of the last week of placebo and test periods were collected by subjects, immediately frozen in specific containers, and delivered to the laboratory. After homogenization, a sample of about 50 g was freeze-dried, weighed and 200 mg of dry sample were analysed for bile acid concentration after ethanol extraction, DEAE-Sephadex A-25 chromatography, methylation and silanization, according to Korpela *et al* (1986), using cholanic acid as internal standard. Analyses were performed using a Varian mod. 3300 gas-chromatograph fitted with FID detector, split/splitless injector and a SPB-1 capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness; Supelco, Bellafonte, USA). Concentrations of the individual bile acids were then reported to the initial faecal specimen and expressed as μmoles excreted over the 24 h.

Breath hydrogen day profile
On three occasions, the volunteers were given a breakfast containing RTE cereals and were followed for the next 13 h to assess colonic fermentation before and after adaptation to inulin. Placebo cereal was given once at baseline, and test cereal was given twice, at the end of placebo period and at the end of test period. Fasting, and at hourly intervals for 13 h after breakfast, forced end-expiratory samples of alveolar air were collected by the subjects using a dedicated plastic bag (GaSampler, Quintron, Milwaukee, WI, USA) into 30 ml plastic syringes with a three-way stopcocks, and analysed for hydrogen concentration with a clinical analyser (Quintron, mod. CM2, Milwaukee, WI, USA). All subjects consumed the same lunch containing few fermentable carbohydrates (white bread, roast-beef, mayonnaise, clear fruit juice) on each of the test days 5 h after the start of breakfast. In addition, they were asked to abstain from cigarette smoking, sleeping and intense physical activity throughout the test day to avoid interference with the H_2 breath test (Thompson *et al*, 1985). Incremental areas under the curve (IAUC) were calculated using the trapezoidal rule.

Glucose tolerance test
Another group of seven healthy volunteers (four females and three males, 28.8 ± 0.8 y, BMI 25.1 ± 1.2 kg/m^2) were enrolled to test the acute effect of cereals on glucose metabolism. After an overnight fast they received, on different mornings and in random order, isoglucidic amounts of test or placebo cereals or bread (40 g of total carbohydrates), plus 200 ml 2% fat milk. Finger prick blood samples were obtained with Glucolet lancets after fasting and at 15, 30, 45, 60, 90, 120, 150 and 180 min after the start of breakfast for the measurement of whole blood glucose with an automatic analyser (Yellow Spring Inst. model 2300 STAT, Yellow Spring, OH, USA). Incremental areas under the 180 min blood glucose curves were used to compare glucose responses of the breakfasts and to calculate the glycaemic index (GI) (Brighenti *et al*, 1995) of placebo and test cereals.

Statistics

Results are expressed as mean ± s.e.m. Differences in glycaemic indices and bile acid excretion between the placebo and test cereals, and in IAUCs of the breath hydrogen day profile at the beginning and end of inulin supplementation were assessed by paired Student's *t*-test (two tail). Blood lipids, bacterial counts, faecal SCFA and L-lactate concentration values at the different time points were submitted to repeated measures analysis of variance (RM-ANOVA), followed, when appropriate, by analysis of covariance (RM-ANCOVA) using changing covariates. Specific differences among treatments were evaluated by applying the Tukey honest significant difference *post-hoc* test. Univariate relationships between blood lipids and faecal composition were evaluated by Pearson's regression analysis, pooling together the data for different treatments. Analyses were performed on a PC using the StatSoft Statistica Package for Windows (release 4.5, StatSoft Inc., Tulsa, OK, USA).

Results

Each participant completed both limbs of the study. All cereals were eaten and the same level of physical activity was maintained throughout the placebo and test periods. Body weight did not change during the study (basal 78.4 ± 4.8; placebo 78.3 ± 4.3; test 78.4 ± 4.2 kg; ns).

Dietary and intestinal habits
Dietary habits slightly changed from basal in that the calories consumed at breakfast increased by 69.4 and 75.6% during the placebo and test periods respectively ($P < 0.02$) (Table 1). No other dietary changes could be appreciated, especially for nutrients known to affect lipid levels, namely alcohol, soluble fibre, simple sugars, cholesterol, saturated and polyunsaturated fatty acids. Intestinal habits were not affected by test or placebo cereals. Some increase in flatulence was occasionally reported after consuming the test cereal but this side effect was well tolerated. The test did not induce abdominal pain, increase faecal weight or number of bowel movements compared to the basal and placebo (Table 2). One subject was not able to provide the faecal specimen within 1 h from defecation at

Table 1 Intakes of nutrients that can affect lipid levels at basal and after placebo and test periods ($n = 12$)

	Basal	*Placebo*	*Test*
Energy (MJ/d)			
Total	(8.46 ± 0.63	9.54 ± 0.46	9.40 ± 0.70
	(5.88 –11.72)	(7.08 –11.75)	(6.76 –13.99)
From breakfast[a]	0.79 ± 0.22	1.34 ± 0.06	1.39 ± 0.07
	(0.00 – 1.96)	(1.14 –1.69)	(1.14 –1.89)
From alcohol	3.47 ± 1.4	2.45 ± 1.13	3.71 ± 1.54
(% of total)	(0.00 –12.15)	(0.00 –11.97)	(0.00 –15.06)
Lipids (g/d)			
Total	72.3 ± 7.7	82.6 ± 4.3	80.7 ± 8.5
	(37.8 –115.2)	(55.5 –103.0)	(39.0 –136.4)
Saturated	23.6 ± 3.4	25.4 ± 1.8	26.4 ± 3.1
	(11.3 –43.3)	(16.7 –35.0)	(14.6 –40.9)
Polyunsaturated	9.3 ± 1.2	9.5 ± 0.6	9.7 ± 1.0
	(6.0 –17.0)	(7.0 –12.2)	(3.9 –15.3)
Cholesterol (mg/d)	243.9 ± 42.6	243.1 ± 32.2	257.2 ± 45.0
	(83.6 –432.0)	(94.5 –460.0)	(88.1 –462.0)
Simple sugars (g/d)	89.2 ± 12.4	80.7 ± 9.5	79.7 ± 11.3
	(28.7 –134.1)	(44.0 –135.0)	(34.2 –144.3)
Dietary fibre (g/d)			
Total	18.5 ± 1.5	20.4 ± 1.4	19.5 ± 2.2
	(12.4 –30.0)	(11.7 –27.7)	(11.7 –36.1)
Soluble	6.6 ± 0.7	7.5 ± 0.5	7.2 ± 0.8
	(4.4 –11.7)	(4.3 –9.8)	(4.3 –12.2)

RM-ANOVA: [a]F (2,18) = 5.55, $P = 0.0132$; basal different from placebo and test $P < 0.05$.

the end of the test period, one subject did not collect a complete 24 h specimen at the end of placebo and test periods, and three subjects did not provide enough samples for acid analysis at the end of the placebo and test periods, and were thus excluded from the relative calculations.

Faecal analyses

The results of pH and bacteria counts for the 11 volunteers that provided the samples are shown in Table 3. The pH did not change between treatments. Compared to basal, total facultative anaerobes were lower at the end of test, and clostridia were higher at the end of wash-out. Bifidobacteria tended to be higher at the end of the test period, and this difference became significant, compared to placebo, after controlling for total anaerobes as the changing covariate ($P < 0.05$). The results of faecal acid concentration for the nine volunteers that provided the samples are shown in (Table 4). The concentration of different SCFAs calculated on wet weight basis tended to be lower, though not significantly, at the end of test compared to all other treatments, whereas L-lactate at the end of test was more than twice that of placebo ($P < 0.05$) and more than three times that of Basal and wash-out ($P < 0.005$). The total excretion of individual and total bile acids over the day at the end of placebo and test periods for the 11 subjects that provided all samples are shown in Table 5. Total bile acid excretion was not different between the two treatments. Primary bile acid increased, and secondary bile acid decreased after test compared to placebo, even though the differences were not statistically significant due to the large variability among individuals.

Table 2 Intestinal habits at basal and after placebo and test periods.

	Basal	*Placebo*	*Test*
Faecal weight (g/d)	168.6 ± 15.6 (101.0 –275.0)	159.3 ± 17.6 (61.0 –265.0)	155.6 ± 13.8 (93.0 –220.0)
Faecal dry weight (%)	23.5 ± 2.3 (8.99 –34.7)	21.38 ± 1.7 (11.5 –29.0)	21.11 ± 2.0 (12.6 –34.6)
Bowel movements/d	1.2 ± 0.1 (1.0 –2.3)	1.1 ± 0.1 (1.0 –1.3)	1.3 ± 0.1 (1.0 –1.6)

Blood lipids

The results of the lipid study for all the 12 volunteers are shown in (Table 6). The treatment had an affect on total cholesterol and TAG concentrations: test being significantly lower than basal for cholesterol and lower than basal and placebo for TAG ($P < 0.05$). Interestingly, the TAG levels remained significantly low, compared to the basal value ($P < 0.05$), after one month of cessation of inulin supplementation (wash-out) when all other parameters had returned to the basal level. From regression

Table 4 SCFAs in the periods of the study ($n = 9$)

	Basal (μmmol/g ww)	*Placebo* (μmol/g ww)	*Test* (μmol/g ww)	*Wash-out* (μmol/g ww)
L-lactate[a]	1.93 ± 0.53 (0.00 –4.44)	3.21 ± 1.25 (0.00 –12.21)	7.39 ± 1.03 (3.55 –12.77)	2.06 ± 1.54 (0.00 –14.21)
Acetate	39.49 ± 6.70 (15.79 –72.42)	42.93 ± 5.68 (14.17 –6.76)	33.26 ± 3.66 (15.69 –50.94)	48.70 ± 8.32 (9.46 –85.81)
Propionate	16.46 ± 3.80 (7.79 –44.71)	14.87 ± 2.41 (5.12 –30.69)	11.99 ± 2.66 (5.18 –30.95)	16.38 ± 2.99 (3.29 –32.66)
Butyrate	16.25 ± 2.60 (6.95 –30.37)	15.28 ± 2.57 (3.84 –28.73)	12.82 ± 2.34 (2.94 –22.27)	18.62 ± 2.84 (3.28 –29.54)

RM-ANOVA: [a]F (3,24) = 6.65, $P = 0.0020$; test different from basal and wash-out $P < 0.005$; from placebo $P < 0.05$

Table 5 Bile acid excretion after placebo and test periods

	Placebo μmol/d	*Test* μmol/d
Cholic acid	2.78 ± 6.16 (0.00 –19.90)	152.82 ± 324.26 (0.00 –958.90)
Chenodeoxycholic acid	8.44 ± 11.34 (0.00 –34.13)	93.27 ± 180.69 (0.00 –455.64)
Deoxycholic acid	299.35 ± 359.22 (0.00 –1099.33)	195.49 ± 181.92 (4.49 –539.49)
Lithocholic acid	229.40 ± 191.12 (30.43 –640.08)	140.02 ± 78.63 (66.38 –334.15)
Total bile acids	544.11 ± 536.10 (30.43 –1760.11)	603.14 ± 654.44 (87.71 –2138.53)
Total primary bile acids	11.22 ± 16.21 (0.00 –54.03)	246.09 ± 501.32 (0.00 –1414.99)
Total secondary bile acid	532.88 ± 542.75 (30.43 –1760.11)	357.05 ± 234.18 (83.45 –783.65)

Table 3 Faecal bacteria counts and pH in the periods of the study ($n = 11$)

	Basal (log CFU/g dw)	*Placebo* (log CFU/g dw)	*Test* (log CFU/g dw)	*Wash-out* (log CFU/g dw)
Bacterial count				
Total facultative anaerobes[a,b]	9.29 ± 0.20 (8.17 –10.04)	9.09 ± 0.21 (8.49 –10.55)	8.52 ± 0.25 (7.33 –10.39)	9.01 ± 0.23 (7.81 –10.12)
Total anaerobes	11.19 ± 0.34 (9.23 –12.48)	11.77 ± 0.10 (11.22 –12.20)	11.64 ± 0.10 (11.05 –12.06)	11.56 ± 0.07 (11.22 –11.87)
Bifidobacteria[c]	10.74 ± 0.10 (10.28 –11.30)	10.66 ± 0.11 (9.88 –11.04)	10.99 ± 0.11 (10.48 –11.57)	10.69 ± 0.09 (10.30 –11.21)
Bacteroides	10.51 ± 0.20 (9.52 –11.42)	11.00 ± 0.13 (10.17 –11.61)	10.75 ± 0.14 (9.98 –11.43)	10.89 ± 0.06 (10.54 –11.24)
Clostridia[d]	7.23 ± 0.54 (4.15 –9.78)	8.70 ± 0.45 (6.16 –9.81)	8.82 ± 0.26 (7.56 –10.09)	8.91 ± 0.44 (5.15 –10.27)
Coliforms	8.83 ± 0.24 (7.59 –9.85)	8.42 ± 0.21 (7.60 –9.86)	7.98 ± 0.27 (6.48 –9.19)	8.31 ± 0.33 (6.59 –10.16)
pH	6.5 ± 0.2 (5.5 –7.5)	6.8 ± 0.2 (6.0 –7.5)	6.3 ± 0.2 (5.5 –7.5)	6.8 ± 0.1 (6.0 –7.5)

RM-ANOVA: [a]F (3,30) = 4.22, $P = 0.0133$; test different from basal $P < 0.01$. [d]F (3,30) = 3.49, $P = 0.0278$; wash-out different from basal $P < 0.05$.
RM-ANCOVA (total anaerobes): [b]F (3,27) = 3.11, $P = 0.0492$; test different from basal $P < 0.01$. [c]F (3,27) = 3.39, $P = 0.0322$; test different from placebo $P < 0.05$.

Table 6 Plasma lipids in the periods of the study ($n = 12$)

	Basal (mmol/l)	*Placebo (mmol/l)*	*Test (mmol/l)*	*Wash-out (mmol/l)*
Cholesterol				
Total[a]	4.24 ± 0.22	4.10 ± 0.23	3.89 ± 0.19	4.14 ± 0.16
	(2.97–5.17)	(2.61–5.19)	(2.38–4.73)	(3.13–5.01)
LDL	2.58 ± 0.21	2.48 ± 0.20	2.31 ± 0.21	2.53 ± 0.17
	(1.42–3.64)	(1.11–3.36)	(1.01–3.18)	(1.60–3.28)
HDL	1.26 ± 0.04	1.23 ± 0.06	1.30 ± 0.08	1.33 ± 0.06
	(0.93–1.47)	(0.78–1.68)	(0.93–1.83)	(1.01–1.60)
Total/HDL ratio	3.41 ± 0.21	3.39 ± 0.21	3.14 ± 0.26	3.19 ± 0.18
	(2.30–4.65)	(2.02–4.48)	(1.84–4.50)	(2.19–4.29)
Triacylglycerols[b]	0.84 ± 0.09	0.84 ± 0.07	0.61 ± 0.05	0.63 ± 0.04
	(0.51–1.63)	(0.45–1.34)	(0.40–0.97)	(0.46–0.89)

RM-ANOVA: [a]$F(3,33) = 3.11$, $P = 0.0394$; test different from basal $P < 0.05$; [b]$F(3,33) = 5.46$, $P = 0.0037$; test and wash-out different from basal and placebo $P < 0.05$.
LDL = low-density lipoprotein, HDL = high density lipoprotein.

analysis none of the faecal organic acids results significantly correlated to TAG levels, whereas a weak but significant positive association was found for butyrate with total and LDL cholesterol and a negative association for propionate with HDL cholesterol (Table 7).

Bile acid excretion over 24 h showed stronger relationships with blood lipids (Table 8). In particular, litocholic acid and total secondary bile acids showed a significant direct relationship with total cholesterol ($P < 0.05$) and triacylglycerols ($P < 0.001$) and an inverse relationship with total/HDL cholesterol ratio ($P < 0.05$), whereas primary bile acids did not show any significant correlation. Blood lipids were also correlated with bacteria count (Table 9). In particular, bifidobacteria were negatively correlated to TAG, as well as total anaerobes. Bifidobacteria were also positively correlated to HDL cholesterol and, as a consequence, negatively correlated to the total/HDL cholesterol ratio. Finally, total facultative anaerobes were negatively correlated to total cholesterol.

Breath hydrogen and glycaemic response

Placebo cereal gave virtually no increase in breath hydrogen concentrations at any time point (IAUC 78 ± 26 ppm·h), whereas after test cereal the day profile showed a marked increase in breath hydrogen concentration, starting from 2 h after breakfast, reaching a maximum around 8 h and then decreasing almost to basal levels by hour 13 (Figure 1). Although the values were somewhat lower for the study performed at the end of test period compared to that performed at the beginning, there was no significant difference between the two trials with cereals containing inulin (IAUC 311 ± 51 ppm·h at the beginning *versus* 280 ± 41 ppm · h at the end; n.s.). The glycaemic responses of test and placebo cereals were virtually the same, and the calculated glycaemic indices were very high for starchy foods (placebo 140.3 ± 43.3; test 130.5 ± 30.6% of white bread) (Figure 2).

Discussion

This study was planned to test the effects of consumption of a RTE cereal containing inulin on lipid metabolism and on colonic milieu in healthy young men. The study design was subject-blinded but not randomized, and we are aware that this design may introduce biases due to the lack of control

Table 7 Correlation coefficients between independent variables (faecal concentration of organic acids) and dependent variables (serum lipids). Data points are from nine subjects considered over four experimental times (total $n = 36$), significance levels are reported as P

	Total cholesterol	*LDL cholesterol*	*HDL cholesterol*	*Total/HDL ratio*	*Triacylglycerols*
L-Lactate	−0.1900	−0.1373	−0.1665	0.0396	−0.0192
	$P = 0.267$	$P = 0.425$	$P = 0.332$	$P = 0.819$	$P = 0.911$
Acetate	−0.0454	0.0369	−0.2082	0.0903	−0.0263
	$P = 0.793$	$P = 0.831$	$P = 0.223$	$P = 0.600$	$P = 0.879$
Propionate	−0.2366	−0.1185	−0.3863	0.1150	0.0431
	$P = 0.165$	$P = 0.491$	$P = 0.020$	$P = 0.504$	$P = 0.803$
Butyrate	0.3339	0.3405	−0.0473	0.2534	0.2017
	$P = 0.047$	$P = 0.042$	$P = 0.784$	$P = 0.136$	$P = 0.238$

LDL = low-density lipoprotein, HDL = high density lipoprotein.

Table 8 Correlation coefficients between independent variables (daily excretion of bile acids) and dependent variables (serum lipids). Data points are from eleven subjects considered over two experimental times (placebo and test) total $n = 22$), significance levels are reported as P

	Total cholesterol	*LDL cholesterol*	*HDL cholesterol*	*Total/HDL ratio*	*Triacylglycerols*
Lithocholic acid	0.4736	0.4593	−0.2527	0.5149	0.7265
	$P = 0.026$	$P = 0.032$	$P = 0.256$	$P = 0.014$	$P = 0.001$
Deoxycholic acid	0.3755	0.3511	−0.1631	0.3922	0.6116
	$P = 0.085$	$P = 0.109$	$P = 0.468$	$P = 0.071$	$P = 0.002$
Chenodeoxycholic acid	0.0707	0.1278	−0.1670	0.1542	0.0530
	$P = 0.754$	$P = 0.571$	$P = 0.458$	$P = 0.493$	$P = 0.815$
Cholic acid	0.0773	0.1200	−0.1318	0.1310	0.0620
	$P = 0.732$	$P = 0.595$	$P = 0.559$	$P = 0.561$	$P = 0.784$
Total bile acids	0.3338	0.3528	−0.2351	0.3965	0.4913
	$P = 0.129$	$P = 0.107$	$P = 0.292$	$P = 0.068$	$P = 0.020$
Total primary bile acids	0.0750	0.1229	−0.1446	0.1395	0.0588
	$P = 0.740$	$P = 0.586$	$P = 0.521$	$P = 0.536$	$P = 0.795$
Total secondary bile acids	0.4246	0.4048	−0.2068	0.4537	0.6769
	$P = 0.049$	$P = 0.062$	$P = 0.356$	$P = 0.034$	$P = 0.001$

LDL = low-density lipoprotein, HDL = high density lipoprotein.

Table 9 Correlation coefficients between independent variables (faecal bacteria counts) and dependent variables (serum lipids). Data points are from eleven subjects considered over four experimental times (total $n=44$), significance levels are reported as P

	Total cholesterol	*LDL cholesterol*	*HDL cholesterol*	*Total/HDL ratio*	*Triacylglycerols*
Bifidobacteria	−0.2234	−0.2794	0.3147	−0.3830	−0.3110
	$P=0.145$	$P=0.066$	$P=0.037$	$P=0.010$	$P=0.040$
Clostridia	0.0577	0.1275	−0.1417	0.1317	−0.0919
	$P=0.710$	$P=0.410$	$P=0.359$	$P=0.394$	$P=0.553$
Total facultative anaerobes	−0.3257	−0.2746	−0.1791	−0.1428	−0.0643
	$P=0.031$	$P=0.071$	$P=0.245$	$P=0.355$	$P=0.678$
Coliforms	−0.1143	−0.1542	0.1452	−0.2026	−0.0595
	$P=0.460$	$P=0.318$	$P=0.347$	$P=0.187$	$P=0.701$
Anaerobes	−0.2374	−0.1691	−0.0124	−0.1727	−0.3729
	$P=0.121$	$P=0.272$	$P=0.936$	$P=0.262$	$P=0.013$
Bacteroides	−0.1466	−0.0722	−0.0894	−0.0532	−0.2616
	$P=0.342$	$P=0.641$	$P=0.564$	$P=0.732$	$P=0.086$

LDL = low-density lipoprotein, HDL = high density lipoprotein.

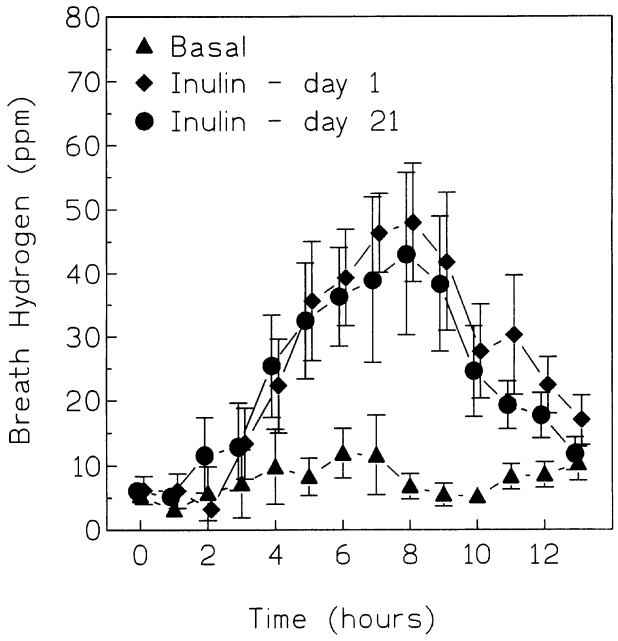

Figure 1 Daily breath-H_2 profiles after consumption of Placebo RTE-cereals (basal) and at the beginning (inulin−day 1) and at the end (inulin−day 21) of test cereal.

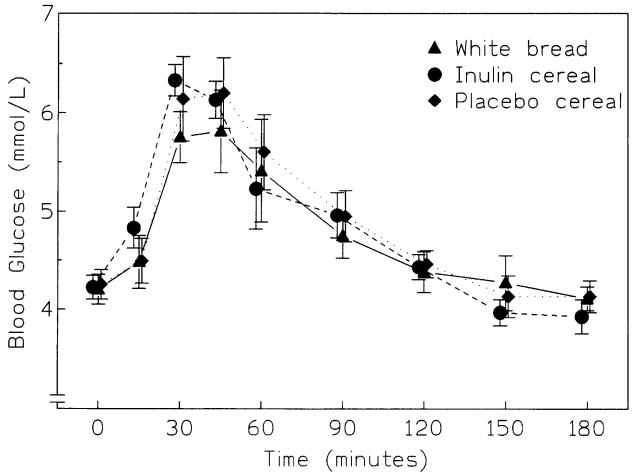

Figure 2 Glycemic responses after consumption of isoglucidic loads of white bread and of test and placebo cereals.

of results. However, in this kind of study where the changes in the intestinal milieu are likely to be subjected to significant carry-over effects, a cross-over design is impractical unless treatments are separated by a wash-out period sufficient to reduce the sequence effect. As no information on a potential carry-over effect due to inulin consumption has been reported in the literature, we chose a design based on a four weeks period with a placebo cereal before inulin administration, followed by a wash-out period of four weeks to evaluate whether a carry-over effect of the treatment exists.

No changes in colonic habits were observed after the consumption of 9 g/d of inulin incorporated in the test cereal. Wet and dry faecal weights, faecal SCFA concentration and pH, stool frequency and gastrointestinal symptoms reported by volunteers after consuming the test cereal were similar to placebo results, despite the fact that inulin markedly enhanced colonic fermentation, as demonstrated by the increase of both breath-H_2 concentration and faecal concentration of L-lactate at the end of the test period. In contrast, Gibson and Roberfroid (1995) found a small but significant increase in faecal weight and nitrogen excretion after supplementation of 15 g/d of inulin or FOS for 15 days. Such differences, which in any case indicate little if any effect of inulin on faecal mass, could be due to different amounts of fibre in the background diet.

If the effects of inulin on faecal excretion are unlikely to be relevant at these levels of intake, its effect on colonic microflora composition could be significant. Supplementation with FOS increases bifidobacteria and decreases bacteroides and clostridia in humans and *in vitro* experiments with faecal inocula (Roberfroid *et al*, 1998). Inulin seems to be less effective in changing the microflora profile although an increase in bifidobacteria has been systematically reported (Roberfroid *et al*, 1998). In our study, we found a small but significant effect of inulin in decreasing total facultative anaerobes and in increasing bifidobacteria after adjusting for total anaerobe counts, without changes in other microbial groups. The similarity of hydrogen levels in breath at the beginning and at the end of inulin supplementation, indirectly confirms that there was not substantial adaptation of H_2-producing species to the substrate. However, it should be pointed out that in our young subjects bifidobacteria counts were already high (10.7 log CFU/g d.w.) at the beginning of the study. It is likely that in subjects harbouring a more varied microflora, such as the elderly, the changes in the proportion of microbial groups could be more relevant. The effect of inulin and

FOS on serum lipids has been consistently reported in different animal studies using either rats (Levrat *et al*, 1991, Delzenne *et al*, 1993, Kok *et al*, 1996, Fiordaliso *et al*, 1995) or hamsters (Trautwein *et al*, 1998). However, results in humans are less conclusive. Pedersen *et al*, (1997) found that incorporating 14 g/d of inulin into a low-fat spread in the diet of healthy young females for a period of 4 weeks did not change HDL-cholesterol, LDL-cholesterol or triacylglycerols concentrations. Similarly, Luo *et al* (1996) did not find differences in blood lipid and apo-lipoprotein levels in healthy volunteers consuming 20 g/d of FOS or sucrose incorporated into cookies as three or four snacks per day for four weeks. In contrast, Yamasita *et al* (1984), and Davidson *et al* (1998) found that FOS and inulin affected total and LDL-cholesterol in diabetic and hyperlipidemic subjects.

Therefore, it is not surprising that the extent and the mechanisms by which fermentable oligofructans could affect lipid metabolism are still a matter of debate. The results of the present study suggest that regular consumption of a food containing inulin could influence lipid levels in healthy subjects. In fact, we found a reduction of serum triacylglycerols and, to a lesser extent, of total cholesterol, with no effects on HDL cholesterol at the end of test period compared to basal values. In general, the cholesterol-lowering ability of soluble fibres has been ascribed to the interruption of enterohepatic circulation of bile acids. This mechanism implies an increase in faecal bile acids excretion. In our study, total bile acid excretion was not modified by inulin, although it must be noted that primary bile acids tended to increase, and secondary bile acids to decrease after test compared to placebo. As a consequence, serum cholesterol results were positively and significantly correlated to the daily excretion of secondary bile acids. It is likely that a reduced transformation of primary to secondary bile acids could have been the consequence of the inhibition of 7-α-dehydroxylating activity of the colonic microflora during inulin supplementation. Therefore, the direct relationship of secondary bile acids excretion with serum cholesterol, and even more with serum triacylglycerols, could not have been causal but merely a confounding factor associated with enhanced colonic fermentation stimulated by inulin. Another mechanism by which soluble fibres might reduce serum cholesterol is their effect in reducing cholesterol absorption during transit along the upper gut. However, it is unlikely that this could have been the mechanism of the observed reduction in serum cholesterol in our study, since inulin was fed exclusively during breakfast which was relatively low in dietary fats. Moreover, Ellegard *et al* (1997) demonstrated that excretion of fat, cholesterol and bile acids in the effluents of ileostomy subjects is not affected by feeding 17 g of inulin and FOS when added to a controlled diet.

The regularity of breakfast habits was found to be associated to serum lipids in Swedish male university students, the greater the regularity the lower the lipid levels (Unden *et al*, 1995). In our group of subjects this fact could have played a major role in decreasing serum cholesterol, since most volunteers did not habitually consume more than a cup of coffee in the morning during basal and wash-out periods, and therefore energy intake was significantly increased at breakfast during the two treatment periods. The length of postprandial fasting can affect blood lipids in different ways: firstly, the exposition of liver to portal bile acids negatively affects the rate of cholesterol synthesis; secondly, prolonged fasting leads to higher levels of insulin release after the first meal of the day, even more if the meal is large. It is known that insulin can stimulate cholesterol synthesis by activating the key enzyme of such a pathway, the HMG-CoA reductase (Lala *et al*, 1994). Increase in the meal frequency from 3 to 17 meals per day significantly reduces serum total and LDL cholesterol, probably as a consequence of a reduced hepatic cholesterol synthesis related to the maintenance of low serum insulin levels in the nibbling diet (Jenkins *et al*, 1989). In our study, an improved regularity of breakfast, and consequently a reduction of the length of fasting periods, could have modulated through insulin levels the synthesis of cholesterol. If regularity of breakfast could have played a role in lowering cholesterol levels, the effect on TAG is more difficult to explain.

Kok *et al*. (1996), demonstrated that the reduction of plasma triacylglycerols and VLDL in rats treated with FOS, was due to the reduction of *de novo* fatty acids synthesis in the liver through inhibition of important lipogenic enzymes, such as the glycerol-3-phosphate acyltransferase and the fatty acids synthase. The same authors postulated that modifications of insulin plasma levels evaluated in their study could, at least in part, explain the metabolic effect of FOS. Effects on insulin could also be reinforced by increased levels of portal acetate and propionate derived from colonic fermentation and, in effect, it has been reported that portal concentration of acetate and propionate increase more than two-fold in FOS-treated rats (Kok *et al*. 1996).

In the present study, we assessed in a meal tolerance test that the glucose raising potentials of the two RTE breakfast cereals were equivalent but, unfortunately, we did not analyse plasma insulin or acetate levels. Moreover, none of the faecal organic acids resulted significantly correlated to TAG levels, even though it is possible that faecal concentrations of SCFA could reflect neither SCFA production by colonic fermentation nor SCFA concentration in the portal vein. Therefore, an involvement of colonic fermentation in the mechanisms by which inulin was able to reduce TAG levels in our subjects is, at present, only a speculation.

Conclusions

In conclusion, in this study we observed: (1) a small change in the intestinal milieu toward a more positive profile of both microflora and bile acids; (2) a reduction in blood lipids, and especially triacylglycerols, of young healthy males consuming a generic food containing a small amount of inulin; and (3) a marked carry-over effect on TAG levels after cessation of inulin consumption. Whether these effects are specifically due to inulin, to the way of administration (a carbohydrate food consumed in the morning after an overnight fast), or to a characteristic of the subjects, such as gender (Wolever *et al*, 1996), remains to be clarified.

Acknowledgements—Supported by grant number 95.00773.PF41 from the National Research Council of Italy. This paper was presented in part at the COST 92 Workshop Dietary Fibre and Fermentation in the Colon, Espoo, Finland, 15–17 June 1995.

References

Brighenti F, Pellegrini N, Casiraghi MC, Testolin G (1997): *In vitro* studies to predict physiological effects of dietary fibre. Eur. J. Clin. Nutr. **49** (Suppl. 3), S81–S88.

Davidson MH, Maki KC, Synecki C, Torri SA & Drennan KB. (1998): Effects of dietary inulin on serum lipids in men and women with hypercholesterolemia. *Nutr. Res.* **18**, 503–517.

Delzenne NM, Kok N, Fiordaliso M-F, Deboyser DM, Goethals FM & Roberfroid MB (1993): Dietary fructooligosaccharides modify lipid metabolism in rats. *Am. J. Clin. Nutr*. **57** (Suppl), 820S.

Ellengard L, Andersson H & Bosaeus I (1997): Inulin and oligofructose do not influence the absorption of cholesterol, Ca, Mg, Zn, Fe, or bile acids but increases energy excretion in ileostomy subjects. *Eur. J. Clin. Nutr*. **51**, 1–5.

Fidanza F, Gentile MG & Porrini M (1995): A self-administered semi-quantitative food-frequency questionnaire with optical reading and its concurrent validation. *Eur. J. Epidemiol*. **11**, 163–170.

Finegold SM, Sugihara PT & Suter VL (1971): Use of selective media for isolation of anaerobes from humans. In: *Isolation of anaerobese*. Shapton DA & Board RG, (eds), London: Academic Press. pp 99–108.

Fiordaliso M, Kok N, Desager JP, Goethals F, Deboyser D, Roberfroid M & Delzenne N (1995): Dietary oligofructose lowers triglycerides, phospholipids and cholesterol in serum and very low density lipoproteins of rats. *Lipids* **30**,163–167.

Gibson GR, Beatty ER, Wang X & Cummings JH (1995): Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. *Gastroenterology* **108**, 975–982.

Holdeman LV & Moore WEC (1973). In: *Anaerobic Laboratory Manual*. 2nd edn. Holdeman LV & Moore WEC (eds), Blacksburg VA: Virginia Polytechnic Institute and State University Ananerobe Laboratory.

Jenkins DJA, Wolever TMS, Vuksan V, Brighenti F, Cunnane SC, Rao AV, Jenkins AL, Buckley G, Patten R, Singer W, Corey P & Josse RG (1989): Nibbling versus gorging: metabolic advantages of increased meal frequency. *N. Eng. J. Med.* **321**, 929–934.

Kok N, Roberfroid M, Robert A & Delzenne N (1996): Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. *Br. J. Nutr*. **76**, 881–890.

Korpela JT, Fotsis T & Adlercreutz H (1986): Multicomponent analysis of bile acids by anion exchange and capillary column gas-liquid chromatography: application in oxytetracycline treated subjects. *J. Steroid. Biochem*. **25**, 277–284.

Lala A, Scoppola A, Ricci A, Frontoni S, Gambardella S & Menzinger G (1994): The effects of insulin on plasma mevalonate concentrations in man. *Ann. Nutr. Metab*. **38**, 257–262.

Levrat M-A, Remesey C, Demigné C (1991): High propionic fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. *J. Nutr*. **12**1, 1730–1737.

Luo J, Rizkalla SW, Alamowitch C, Boussairi A, Blayo A, Barry J-L, Laffitte A, Guyon F, Bornet FRJ & Slama G (1996): Chronic consumption of short-chain fructooligosaccharides in healthy subjects decrease basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. *Am. J. Clin. Nutr*. **63**, 939–945.

Mevissen-Verhage EAE, De Vos NM, Harmesen van Amerongen WCM, Marcelis JH (1982): A selective medium for detection and enumeration of clostridia in human faeces. *Antonie van Leeuwenhoek* **48**, 205–206.

Pedersen A, Sandstrom B & Van Amelsvoort JMM (1997): The effect of ingestion of inulin on blood lipids and gastrointestinal symptoms in healthy females. *Br. J. Nutr*. **78**, 215–222.

Roberfroid M (1993): Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *CRC Crit. Rev. Food. Sci. Nutr*. **33**, 103–148.

Roberfroid MB, Van Loo JAE, Gibson GR (1998): The bifidogenic nature of chicory inulin and its hydrolysis products. *J. Nutr*. **128**, 11–19.

Scheppach WM, Fabian CE & Kasper HW (1987): Fecal short chain fatty acid (SCFA) analysis by capillary gas-liquid chromatography. *Am. J. Clin. Nutr*. **46**, 641–646.

Teraguchi S, Uehara M, Ogasa K, Mitsuoka T (1978): Enumeration of bifidobacteria in dairy products. *Jap. J. Bact*. **33**, 753–761.

Thompson DG, Binfield P, De Belder A, O'Brien JB, Warren S & Wilson M (1985): Extra intestinal influences on exhaled hydrogen breath measurements during the investigation of gastrointestinal disease. *Gut* **26**, 1349–1352.

Trautwein EA, Radunz E, Rieckhoff D & Erbersdobler HF (1998): Effects of increasing doses of dietary invulin on cholesterol and bile acids metabolism in hamster. In: *Functional properties of non-digestible carbohydrates*, Guillon F *et al*. (eds), Nantes: Imprimerie Parentheses. pp 132–133.

Unden AL, Krakau I, Hogbom M & Romanus-Egerborg I (1995): Psychosocial and behavioral factors associated with serum lipids in university student. *Soc. Sci. Med*. **41**, 915–922.

Van Loo J, Coussement P, De Leenheer L, Hoebregs H & Smits G (1995): On the presence of inulin and oligofructose as natural ingredients in the Western diet. *CRC Crit. Rev. Food. Sci. Nutr*. **35**, 525–552.

Vanhoof K & De Schrijver R (1995): Effect of unprocessed and baked inulin on lipid metabolism in normo- and hypercholesterolemic rats. *Nutr. Res*. **15**, 1637–1646.

Wolever TMS, Fernandes J & Rao AV (1996): Serum acetate:propionate ratio is related to serum cholesterol in men but not in women. *J. Nutr*. **126**, 2790–2797.

Yamashita K, Kawai K & Itakura M (1984): Effects of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. *Nutr. Res*. **4**, 961–966.