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ORIGINAL ARTICLE

Long-term gastrointestinal tolerance of NUTRIOSE®FB in healthy men

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Objective: To determine the gastrointestinal (GI) tolerance of NUTRIOSE®FB in men.
Design: A randomized, placebo-controlled, parallel, double-blind study.
Setting: The metabolic ward of TNO Quality of Life.
Subjects: Forty-eight subjects started the study: 16 men participated in one of the three treatments. Subjects consumed either 22.5 g of pure maltodextrin (Glucidex®6), or 30 or 45 g of the dextrin NUTRIOSE®FB daily for 4–5 weeks. Forty-three subjects completed the study (age: 34.7 ± 8.2 years; BMI $24.9 \pm 3.3\,kg\,m^2$).
Measurements: Tolerance of NUTRIOSE®FB was examined with a GI complaints questionnaire; effectiveness on colonic flora was examined by faecal analysis; fermentation by breath hydrogen excretion measurement. Furthermore, the effect on body weight (BW), energy intake and blood parameters were examined in the study.
Results: Both doses of NUTRIOSE®FB were very well tolerated and GI complaints hardly differed from the placebo treatment. No diarrhoea was reported due to NUTRIOSE®FB supplementation. In the course of the study, some habituation and adaptation of GI symptoms were found. Fermentation and faecal characteristics (pH and enzyme activity) were significantly positively affected with NUTRIOSE®FB treatment. Body weight in both NUTRIOSE®FB groups remained stable over time, although the placebo-treated group showed a small increase in BW (Δday_{35-1} 0.8 ± 1.0 kg) ($P = 0.07$). However, total food intake and macronutrient composition of the diet remained the same throughout the study. No significant differences were found between the three treatment groups in hunger and satiety scores and food preferences.
Conclusions: Long-term supplementation of 30 or 45 g of the dextrin NUTRIOSE®FB per day was well tolerated, and may act as a pre-biotic supplement.
Sponsorship: TNO Quality of Life was assigned by Roquette Frères to perform the study.
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Introduction

It is well known that fermentation of carbohydrates that escape digestion exert a number of effects that are beneficial for (colonic) health: growth of bacteria in the colon optimizing intestinal environment, an increase in stool volume, a shorter intestinal transit time, production of short-chain fatty acids (SCFA) and a decrease of colonic pH (Kritchevsky, 1988). This may lead to changes in the composition of bile acids in the colon and in the activity of faecal enzymes, some of them may in turn play a role in the pathogenesis of colon cancer (Reddy *et al*., 1992) and others could be considered as good for health. The presence of carbohydrate fermentation supplies the bacteria their energy, which should otherwise be obtained from protein fermentation. The subsequently formed metabolites are unhealthy and related to carcinogenesis.

Besides colonic health and a possible anticarcinogenic effect, dietary fibres are studied because of their satiating effect. Dietary fibres have been shown to increase satiety feelings and to reduce food intake (Pasman *et al*., 1997). With long-term supplementation of NUTRIOSE®FB, also effects of dietary fibre (soluble and insoluble) on body weight (BW) and body composition may be of interest. After

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fibre consumption, flattening of blood levels of insulin and glucose have been reported, resulting in decreased appetite (Albrink, 1978; Hamilton and Anderson, 1992; Rössner, 1992) and less food intake (Evans and Miller, 1975; Levine *et al*., 1989; Cybulski *et al*., 1992; DeLargy *et al*., 1995; Pasman *et al*., 1997). It is therefore interesting whether NUTRIOSE®FB in the long term, by reducing energy intake, affects body weight and body composition. In epidemiological studies, people eating a relatively high dose of dietary fibre have lower BMIs than their less fibre eating counterparts (Lovejoy and DiGirolamo, 1992; Spiller, 1993; Alfieri *et al*., 1995; Wynder *et al*., 1996). Because it is not clear whether this is causative or not, an intervention with dietary fibre might give some insight into this mechanism.

In the present study, a dextrin, NUTRIOSE®FB, obtained from wheat starch has been studied. In contrast to a normal maltodextrin, NUTRIOSE®FB is not completely hydrolysed and absorbed in the small intestine, because of a higher percentage of α-1,6 linkages and the presence of non-digestible glucoside linkages (e.g. α-1,2 and α-1,3). Previous results from rat experiments showed that about 75% of the product is resistant to the enzymatic glucidolytic degradation. Supplementation in humans revealed that 87% of the supplemented NUTRIOSE®FB is digested and fermented (van den Heuvel *et al*., 2005). Therefore, NUTRIOSE®FB could be considered as a soluble fibre. Some soluble dietary fibres induce intestinal discomfort owing to the increase of water content related to the osmotic power of these molecules and owing to the production of gases. Compared to other oligosaccharides, NUTRIOSE®FB may show a better tolerance as it is partly digested and absorbed in the small intestine and about one-fifth of NUTRIOSE®FB is excreted in the faeces, as is supported with results of human studies. A daily dose of up to 80–100 g of NUTRIOSE®FB is well tolerated in man (Coudray *et al*., 2003; van den Heuvel *et al*., 2004).

To examine the tolerance of long-term NUTRIOSE®FB consumption, the two best-tolerated doses from a previous study (van den Heuvel *et al*., 2004) have been chosen for further investigation. An optimum of tolerance and effectiveness on colon flora is thought to be realized with 30–45 g of NUTRIOSE®FB. The effect of NUTRIOSE®FB supplementation was studied on subjective evaluation of gastrointestinal (GI) (dis)comfort, defecation and colon flora. Because of the long-term supplementation, the satiating effect of NUTRIOSE®FB affecting BW, body composition, hunger and satiety feelings, food preferences and energy intake will be examined as well.

Materials and methods

Subjects

Male subjects aged between 20 and 45 years were recruited from the pool of volunteers of TNO Quality of Life and by advertisements in local newspapers. After signing the informed consent forms, health was assessed at pre-study screening. This included an interview on medical history, physical examination and routine laboratory tests on blood and urine sampled after an overnight fast. Subjects needed to meet the inclusion and exclusion criteria related to the parameters of the study (age between 20–45 years; BMI $\leqslant 31\,kg\,m^2$; regular eating pattern; regular defecation pattern; no metabolic, endocrine, intestinal disorder or a medical history related to the study outcome parameters; no lactose malabsorption; no colour blindness). From 1 month before the start of the study, subjects were instructed not to use antibiotics or laxatives.

In total, 48 men started the study. The participants were randomly assigned to one of the three treatments, with randomization restricted by age, BMI and smoking habit. The baseline characteristics of the 43 subjects who completed the study are presented in Table 1. The study was approved by the Medical Ethics Committee of TNO (5 September 2000) and conducted according to the ICH Guideline for Good Clinical Practice from October 2000 to January 2001.

Study design

The study was performed according to a randomized, parallel, placebo-controlled, multiple dose and double-blind design.

Study substances

The study substance tested, NUTRIOSE®FB, is a purified glucose polymer from wheat starch from Roquette Frères, Lestrem, France. After purification, it has been modified into

Table 1 Baseline characteristics of the participating subjects (mean ± s.d.) ($n = 43$)

	Glucidex®6 (n = *13*)	*30 g NUTRIOSE®FB* (n = *14*)	*45 g NUTRIOSE®FB* (n = *16*)
Age (years)	33.8 ± 10.1	35.7 ± 7.6	34.4 ± 7.4
Systolic blood pressure (mm Hg)	131 ± 15	138 ± 18	138 ± 12
Diastolic blood pressure (mm Hg)	80 ± 8	84 ± 7	85 ± 10
Body weight (kg)	80.4 ± 11.8	82.1 ± 12.0	86.0 ± 12.9
BMI (kg/m^2)	24.5 ± 2.9	24.9 ± 3.2	25.3 ± 3.7
Total cholesterol (mmol/l)	5.3 ± 1.4	5.6 ± 1.2	5.3 ± 1.0
LDL-cholesterol (mmol/l)	3.5 ± 1.2	3.7 ± 1.0	3.5 ± 0.8
HDL-cholesterol (mmol/l)	1.2 ± 0.4	1.3 ± 0.4	1.2 ± 0.3

Table 2 Description of NUTRIOSE®FB and Glucidex®6

	NUTRIOSE®FB	*Glucidex®6*
Reducing sugars (%)	2.3	5.7
H_2O content (%)	3.5	3.8
Proteins (%)	<0.5	–
Ash (%)	<0.5	–
Free glucose (%)	<0.1	–
Total dietary fibre (%)	53	<2
α-1,4 linkages (%)	76	95
α-1,6 linkages (%)	24	5
Degree of polymerization	15.3	21.5
Molecular weight (g)	5344	33 075
Molecular weight (*n*)	2480	3480
Weight range index	2.1	9.5
Energy value (kcal/g)	2	4

a polysaccharide that cannot be digested totally in the GI tract. The placebo used was Glucidex® 6, a pure maltodextrin, which is hydrolysed and absorbed in the small intestine up to at least 95%. The two study substances used are described in detail in Table 2. Both study substances were supplied to the subjects as a powder. To avoid colour and taste differences, caramel colour and aspartame were added to the substances in sufficient quantities to reach the same organoleptic characteristics.

Study treatment

In the run-in week, all subjects were supplied with 22.5 g of Glucidex®6 per day. The different treatments after the run-in week were:

- 15 g of NUTRIOSE®FB daily during the first week of the study, followed by 4 weeks of 30 g of NUTRIOSE®FB daily;
- 22.5 g of NUTRIOSE®FB daily during the first week of the study, followed by 4 weeks of 45 g of NUTRIOSE®FB daily;
- 11.25 g of Glucidex®6 daily during the first week of the study, followed by 4 weeks of 22.5 g of Glucidex®6 daily.

To avoid GI complaints due to an acute increase in oligosaccharides/dietary fibre, subjects were supplied with half the dosage during the first week.

The energy level of the 45 g dose was controlled for with 22.5 g of reference substance Glucidex®6, because Glucidex®6 contained twice as much energy.

Each daily dose was consumed in four portions and was dissolved by the subjects in yoghurt, hot or cold drinks and consumed along the day: at breakfast, during the morning, at lunch and at dinner. In case one dose of the four daily doses was forgotten, the subjects were asked to consume it later on during the same day.

With respect to food intake, subjects were asked to keep their habitual diet. However, because of the study substance probably affecting GI flora, other food products with similar effects were prohibited during the whole study. Therefore, food items containing pre- and pro-biotics and fibre were not allowed (dairy products with added cultures, asparagus, vegetable oysters, artichokes and rye bread). Consumption of some food items was restricted, meaning that subjects were not allowed to consume the product more than once a week and only in small amounts (onions, leak, garlic and bananas).

Study protocol

During the total 6 weeks of study period (run in period: day −7 till day −1; first week half dose of treatment and 4 weeks full treatment dose; day 1 till day 35), subjects came to the metabolic ward weekly to collect study substances (days −7, −1, days 07, 14, 21, and 28). At day −1, day 21 and day 35, subjects came to the metabolic ward of TNO after an overnight fast. After blood sample collection, subjects received a standard breakfast including their study treatment. Three-quarters of the daily dose of the study products were consumed (with breakfast, snack and lunch) at the test day at TNO. On all test days, during the whole day, every half to 1 h, subjects exhaled breath in a hydrogen analysis apparatus and scored hunger and satiety questionnaires. A faecal sample was collected on the test day or the day before at TNO or at home.

After breakfast, consumption anthropometry of the subjects was carried out and a food frequency questionnaire (FFQ) was filled in as well (only on day −1 and day 35).

Study parameters

Blood parameters. Blood was collected after an overnight fast from the antecubital vein, using Vacutainer tubes (10 ml). For serum collection, blood was collected in tubes containing clot activator and centrifuged for 10 min 15–30 min after collection. The centrifuge was adjusted at ca. 2000 g and approximately 4°C. After centrifugation, serum was removed. All samples were stored at −18°C, until analysis. Pre-study clinical chemistry and safety parameters were determined. In study parameters glucose, triacylglycerols (TG) and free fatty acids (FFA) were determined using commercial test kits (Boehringer, Mannheim, Germany) on a Hitachi 911 automatic analyser (Hitachi Instrument Division, Ibaraki-ken, Japan). Insulin was determined using AIA-600 Immunoassay Analysator. Total cholesterol and HDL-cholesterol, apo-a1 and apo-b and triacylglycerol (TG) were determined enzymatically (Roche Diagnostics, Mannheim, Germany). LDL cholesterol (Friedewald *et al*., 1972) and the ratio Total/HDL cholesterol were calculated. Leptin was measured according to the instructions of the standard RIA kit, mainly based on Ma *et al*. (1996).

Blood acetate was measured after blood was deprotonated and subsequently diluted with water. Acetic acid was determined by ion-exclusion chromotography with the Dionex DX500 ion-chromotograph and detected by chemical-suppressed conductivity. The eluent used was 0.4 mmol Heptafluorobuteric-acid/l and the analytical column used was a Dionex IonPac ICE-AS6. Of the sample, 400 μl serum

was mixed with 400 μl acetonitril. This was homogenized and centrifuged for 10 min with 14 000 *g* in an Eppendorf centrifuge. Of the clear supernatant, 200 μl was again diluted with 600 μl water. This was once again homogenized and injected in the Dionex DX500 ion-chromatograph, and measured according to the standard chromatographic procedures (www.dionex.com, application note 107).

Faecal parameters. The faecal collection protocol is performed according to established protocols (van Nuenen *et al*., 2003; Venema *et al*., 2005). Spot faecal samples were collected in a sterile gas-tight bag, in a plastic container containing an Anaerocult® strip to create anaerobic conditions. Of the 3×43 feacal sample collections, some were collected at home. In those cases that samples were collected at home, it was brought to the institute within 2 h and immediately cooled and transferred within half an hour to an anaerobic cabinet, and processed. We have shown that this 2.6 h time-frame between collection and processing in this method does not influence microbial composition or metabolite concentration (unpublished data).

Fat, sterols, lactic acid, pH, water content, wet and dry weight, SCFA and microbiology were determined in (fresh) faeces collected at days −2/−1, day 20/21 and day 34/35.

The bacteria determined in fresh faeces were total anaerobes, bifidobacteria, bacteroides, enterobacteriaceae, lactobacilli, clostridia and enterococci. These anaerobic and aerobic bacteria were measured in homogenized faeces, obtained from 2 g of faeces taken from the centre of the bolus. The faecal suspension, obtained after centrifugation of the faeces with the freezing medium, was transferred to cryotubes and stored in liquid nitrogen for later analysis. The faecal suspension was plated on plates containing different types of medium. After 2 days of incubation at 37°C, the number of colonies was counted.

Wet weight and pH were determined at the beginning of faeces preparation as well.

After processing of the faeces, SCFA and lactate were measured. The SCFAs (acetic, proprionic, butyric, valeric, iso-valeric and iso-burytic acid) were determined in faecal suspensions and measured on a gas chromotograph (GC; Chrompack CP9001, Varian, Bergen op Zoom, The Netherlands) according to the method described by Jouany (1982). The two forms of lactate (D- and L-) were measured enzymatically using a Cobas Mira Plus autoanalyser (Roche) and is based on the principle of conversion of NAD into NADH. Finally, a sub-sample of the faeces was necessary for the determination of fat content and sterol differentiation (campesterol, coprostanol, cholesterol, stigmasterol and β-sitosterol). The fat content of the faeces was performed according to a direct extraction process. The faecal samples were extracted with petroleum ether under reflux. The solvent is evaporated and the mass of the residue was determined and expressed as fat.

The remains of the faecal sample were freeze dried for ca 4 days or until dry. After weighing, the freeze-dried samples were grounded for ca. 1 min to ca. 500 μ parts.

The homogenized freeze-dried faeces were analysed for NUTRIOSE®FB residuals and α-glucosidase [EC 3.2.1.20] and β-glucosidase [EC 3.2.1.21] enzyme activity, as described elsewhere (van den Heuvel *et al*., 2005).

Questionnaires. Subjective evaluation of well being and GI comfort were examined using questionnaires on days −1, 7, 14, 21, 28 and 35. Complaints, compliance and use of medication were questioned on the well-being form. With a GI questionnaire, GI comfort, complaints, occurrence and severity of abdominal symptoms (excess flatus, borborygmi, bloating, abdominal cramps), diarrhoea, number of stools and consistency of stools were evaluated using contiuous and categorical questions over the last 6 days and the last 24 h. When a GI complaint was present, the severity of the complaint was scored on a 100 mm line (visual analogue rating scale: VARS).

Total food intake was investigated using the FFQ (day −1 and day 35). The FFQ was developed in FOFREX (Food Frequency Expert), a computerized system with data from the second Dutch national food consumption survey of 1992 (National Education Board, 1992) and a predefined question matrix. Changes in dietary intake (total energy, protein, fat, carbohydrates, alcohol, dietary fibre) over the past 5 weeks were evaluated.

In addition, the subjects filled in a diary report about the time of ingesting the study substance (compliance), the frequency of defecation and consistency of the stool on each day during the study.

Anthropometry. Body weight was measured on days −1, 21 and 35. Body composition was measured before the actual supplementation started (day −1) and at the end of the study period (day 35) and was determined with the bio-impedance method as described elsewhere (Pasman *et al*., 1997). Body weight minus fat-free mass (FFM) is the calculated fat mass of the body. The body fat percentage was calculated by dividing fat mass by body weight and multiplying the outcome with 100%. Waist and hip circumferences were measured on days −1 and 35.

Kinetic parameters

Breath H_2-excretion test. On days −1, 21 and 35, the breath H_2-excretion tests were performed with an EC60 Gastrolyzer, a portable breath H_2 monitor (Bedfont Instruments, Kent, UK). Breath samples were taken just before breakfast (0) and at 60, 120, 150, 180, 210, 240 (just before lunch), 270, 300, 330, 360, (just before snack) 390, 420, 450 and 480 min after the ingestion of the study substance during breakfast.

Subjects were instructed to exhale as deeply as possible to obtain alveolar air. The subjects exhaled directly into the

apparatus via a mouthpiece. Within each subject, all measurements were done with the same apparatus. The interval between ingestion of NUTRIOSE®FB and the initial sustained rise in breath hydrogen $\geqslant$10 p.p.m. from basal concentrations (the lowest previous concentration) represents the orocaecal transit time (OCTT) (Jorge *et al*., 1994; Rumessen and Gudmand-Høyer, 1998).

Visual analogue rating scales. Food preferences, appetite, meal and snack frequency, and hunger and satiety ratings were scored on VARS. The items were scored at 0, 30, 60, 90, 120, 180, 240 (just before lunch), 300 and at 480 min on days −1, 21 and 35.

Feelings of hunger and satiety were rated by means of a slash on six 100 mm lines labeled as follows:

Appetite for a meal; Appetite for something sweet; Appetite for something savoury; Satiety (fullness); Feeble/weak with hunger and Appetite for a snack. Oral instructions were provided to inform the subjects about the meaning of these terms (Hulshof, 1994).

Statistics

Data are expressed as means ± standard deviations (s.d.) for each treatment. Safety parameters, adverse events and results of compliance are presented descriptively.

Hydrogen excretion, hunger and satiety questionnaires were evaluated by areas under the curve (AUCs), time to reach maximum value (T_{max}) and the maximal value itself (C_{max}).

Colon flora, anthropometry, blood and faeces parameters, FFQ and the AUC-characteristics of hydrogen excretion, and hunger and satiety questionnaires were evaluated on treatment effects.

For these parameters, changes over time were calculated as differences (Δ's) between the test days. These Δ's were evaluated on treatment effects with ANOVA, more specifically using the general linear model procedure (GLM) with treatment as a factor. When data were not normally distributed, data were natural log transformed before GLM. Although Δ's were statistically tested, the values measured are presented in the tables. Significant Δ's are presented in the text.

Statistical analyses were carried out with SAS statistical software (SAS institute Inc., 1989, version 6.12). The level of significance was pre-set at 0.05 (two-sided).

Results

General

The study was completed by 43 subjects: 13 subjects on Glucidex®6 treatment, 14 subjects on the lowest dose of NUTRIOSE®FB and 16 subjects on the highest dose of NUTRIOSE®FB. Five subjects did not complete the study: four subjects dropped out during the run-in week and one subject was ill on day 35.

Compliance of the study substance intake was high; 99.8% of both NUTRIOSE®FB treatments and 99.6% for the Glucidex®6 group.

During the study, no serious adverse event occurred. A total of 113 adverse events (AEs) were reported by 39 subjects; 98 cases (87%) were not related and 15 cases (13%) were possibly related to treatment. Of these 15 related AEs, three AEs were reported during the Glucidex®6 treatment, three AEs for the 30 g of NUTRIOSE®FB and nine AEs for the 45 g of NUTRIOSE®FB/day, mainly more subjects reporting flatulence and one subject complained about functional intestinal disorder (three times). The four dropouts and one non-completer were not study treatment-related.

Blood parameters. All blood parameters measured showed hardly any effect of treatment over time. Only a change in total cholesterol level at day 35 versus day 21 for Glucidex®6 ($\Delta + 0.1 \pm 0.4$ mmol/l) versus 30 g of NUTRIOSE®FB ($\Delta -0.4 \pm 0.5$ mmol/l) differed significantly. For the 30 g of NUTRIOSE®FB a day, the total cholesterol level decreased from 5.8 mmol/l at day 21 to 5.4 mmol/l at day 35. For Glucidex®6, a small increase of 0.1 mmol/l was seen in this period (from 5.3 to 5.4 mmol/l). This difference in treatment effect over time was significant. The total cholesterol level for the highest NUTRIOSE®FB dose was 5.3 mmol/l at day 21 and remained 5.3 mmol/l.

The other blood parameters, glucose, insulin, TG, the cholesterol fractions HDL- and LDL, Apo-A1 and Apo-b, as well as acetate and leptin remained similar during the intervention.

Faecal parameters. Data of the faecal samples analysed on substrates, microbiology and enzymatic activity, are presented in Tables 3–5.

Small changes in the faecal fat fractions, coprostanol, cholesterol and β-sitosterol, were observed (all presented as g/kg dry weight). For both Glucidex®6 and 30 g of NUTRIOSE®FB, on average, an increase was seen from day −1 to day 21 ($+0.7 \pm 4.0$) versus a reduction in coprostanol for 45 g of NUTRIOSE®FB (-4.2 ± 5.9). For days 21–35, both NUTRIOSE®FB treatments revealed different coprostanol concentration changes too: for 30 g of NUTRIOSE®FB, a decrease was seen, Δ_{35-21} of -1.7 ± 3.1, whereas for 45 g of NUTRIOSE®FB an increase was seen, Δ_{35-21} of 1.6 ± 3.1.

At the end of the study, faecal cholesterol Δ_{35-21} for Glucidex®6 of -0.6 ± 1.4 was significantly different from Δ_{35-21} for 30 g of NUTRIOSE®FB of $+0.9 \pm 1.2$.

A clear decrease of 33% in β-sitosterol concentration was found from day −1 to day 21 after treatment with 30 g NUTRIOSE®FB, compared with Glucidex®6 and the highest dose of NUTRIOSE®FB treatment. The decrease of Δ_{21-1}

Table 3 Faecal substrate data (mean ± s.d.)

Parameter	*Day*	*Glucidex®6 (n = 13)*	*30 g NUTRIOSE®FB (n = 14)*	*45 g NUTRIOSE®FB (n = 16)*
Coprostanol (g/kg dry weight)	−1	12.6 ± 2.5	11.9 ± 5.8	14.5 ± 5.9
	21	13.4 ± 4.3[a]	12.7 ± 5.8[b]	10.3 ± 4.6
	35	12.8 ± 4.3	10.7 ± 6.0[c]	12.0 ± 4.3
Cholesterol (g/kg dry weight)	−1	1.9 ± 1.1	3.7 ± 2.9	2.8 ± 1.6
	21	2.2 ± 1.9	2.6 ± 2.3	3.4 ± 3.0
	35	1.7 ± 1.1[d]	3.4 ± 3.0	3.9 ± 3.3
β-Sitosterol (g/kg dry weight)	−1	0.9 ± 0.3	1.5 ± 1.0	1.1 ± 0.4
	21	1.0 ± 0.4[e]	1.0 ± 0.7[b]	1.2 ± 1.0
	35	0.9 ± 0.4	1.3 ± 0.9	1.2 ± 1.1
Total lactate (g/l)	−1	1.9 ± 2.9	0.5 ± 0.9	0.7 ± 0.9
	21	1.1 ± 2.3[e]	0.5 ± 0.8	0.2 ± 0.4
	35	1.0 ± 1.8	0.6 ± 1.2	0.1 ± 0.3
pH	−1	6.6 ± 0.5	6.5 ± 0.5	6.6 ± 0.5
	21	6.6 ± 0.8[a]	6.2 ± 0.7	6.1 ± 0.5
	35	6.5 ± 0.3	6.1 ± 0.4	6.1 ± 0.6
SCFA (mmol/l)	−1	129.2 ± 41.6	119.3 ± 48.8	138.8 ± 38.8
	21	132.7 ± 42.6	154.5 ± 34.4	130.5 ± 38.9
	35	128.1 ± 30.9	131.3 ± 32.2	134.0 ± 30.4

The significant differences found are denoted with a character:
[a]The change over time between day 21 and day -1 differed significantly between Glucidex®6 and 45 g of NUTRIOSE®FB.
[b]The change over time between day 21 and day -1 differed significantly between 30 g of NUTRIOSE®FB and 45 g of NUTRIOSE®FB.
[c]The change over time between day 35 and day 21 differed significantly between 30 g of NUTRIOSE®FB and 45 g of NUTRIOSE®FB.
[d]The change over time between day 35 and day 21 differed significantly between Glucidex®6 and 30 g of NUTRIOSE®FB.
[e]The change over time between day 21 and day −1 differed significantly between Glucidex®6 and 30 g of NUTRIOSE®FB.

Table 4 Faecal microbiology (log transformed data) (mean ± s.d.)

Parameter	*Day*	*Glucidex®6 (n = 13)*	*30 gr NUTRIOSE®FB (n = 14)*	*45 gr NUTRIOSE®FB (n = 16)*
Lactobacilli (log CFU/g faeces)	−1	7.2 ± 1.6	7.6 ± 1.2	7.2 ± 1.4
	21	8.1 ± 1.5	8.4 ± 0.8	7.9 ± 0.8
	35	7.7 ± 1.3	7.4 ± 1.5[a,b]	8.2 ± 1.2
Clostridia (log CFU/g faeces)	−1	6.4 ± 1.2	6.1 ± 1.2	5.9 ± 0.9
	21	6.6 ± 0.7	5.5 ± 1.1	5.8 ± 1.2
	35	6.5 ± 0.7	5.5 ± 0.9	5.6 ± 0.8
Total anaerobes (log CFU/g faeces)	−1	9.8 ± 1.0	10.1 ± 0.3	9.9 ± 0.3
	21	9.8 ± 0.6	9.9 ± 0.5	9.8 ± 0.5
	35	9.9 ± 0.3	9.7 ± 0.5[a]	10.1 ± 0.5

The significant differences found are denoted with a character:
[a]The change over time between day 35 and day −1 differed significantly between 30 g of NUTRIOSE®FB and 45 g of NUTRIOSE®FB.
[b]The change over time between day 35 and day 21 differed significantly between 30 g of NUTRIOSE®FB and 45 g of NUTRIOSE®FB.

Table 5 Faecal enzymatic content and residual branched glucose (mean ± s.d.)

Parameter	*Day*	*Glucidex®6 (n = 13)*	*30 gr NUTRIOSE®FB (n = 14)*	*45 gr NUTRIOSE®FB (n = 16)*
α-Glucosidase (U/g faeces)	−1	1.7 ± 0.7	1.7 ± 0.6	1.8 ± 1.2
	21	1.5 ± 0.5[a,b]	2.5 ± 0.9	3.0 ± 1.2
	35	1.8 ± 1.3[c]	2.8 ± 1.5	3.6 ± 1.7
β-Glucosidase (U/g faeces)	−1	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
	21	0.4 ± 0.2[a,b]	1.3 ± 0.5	1.5 ± 0.5
	35	0.5 ± 0.3[c,d]	1.3 ± 0.6	1.5 ± 0.8
Branched glucose concentration (mg/g faeces)	−1	5.3 ± 2.4	6.0 ± 2.9	6.2 ± 1.8
	21	4.9 ± 2.7[a,b]	47.1 ± 49.4	70.9 ± 51.0
	35	4.9 ± 2.4[c,d]	58.8 ± 64.9[e]	62.3 ± 56.6

The significant differences found are denoted with a character:
[a]The change over time between day 21 and day −1 differed significantly between Glucidex®6 and 30 g of NUTRIOSE®FB.
[b]The change over time between day 21 and day −1 differed significantly between Glucidex®6 and 45 g of NUTRIOSE®FB.
[c]The change over time between day 35 and day −1 differed significantly between Glucidex®6 and 45 g of NUTRIOSE®FB.
[d]The change over time between day 35 and day −1 differed significantly between Glucidex®6 and 30 g of NUTRIOSE®FB.
[e]The change over time between day 35 and day 21 differed significantly between 30 g of NUTRIOSE®FB and 45 g of NUTRIOSE®FB.

-0.5 ± 0.6 with 30 g of NUTRIOSE®FB was significantly different from the other two treatments. In the Glucidex®6 and 45 g of NUTRIOSE®FB-treated groups, on average, the faecal β-sitosterol concentrations remained similar.

Total lactate, pH and SCFA were measured as markers for the degree of acidity of the intestinal environment. Over the first 21 days of the study, significant changes in total lactate were seen between Glucidex®6 and the 30 g of NUTRIOSE®FB treatment: Δ_{21-1} -0.8 ± 3.1 and 0.2 ± 1.0 g/l, respectively. The numerical decrease present for the 45 g NUTRIOSE®FB-treated group over time (day -1 versus day 35) was not statistically significant, probably because a similar change was seen in the reference-treated group as well as the variation of the data.

The pH of the faeces in both NUTRIOSE®FB-treated groups decreased with treatment duration from 6.6 (day -1) to 6.1 (day 35), indicating increased fermentation; for the reference-treated group, pH remained 6.5–6.6. The difference was significant only between the reference group Δ_{21-1} 0.0 ± 0.5 and the 45 g of NUTRIOSE®FB Δ_{21-1} -0.5 ± 0.6. The pH remained stable from day 21 onwards for all groups.

The total sum of the SCFA (acetate, propionate, butyrate, and iso-forms of SCFA) did not show changes in concentration due to time or study substance.

With respect to the microbiological data (log transformed), mainly changes in *Lactobacilli* counts were found, as is presented in Table 5. The statistics performed on the *Lactobacilli* data showed a significant increase in *Lactobacilli* for day 35 versus day –1 for the 45 g NUTRIOSE®FB treatment. On days 21 and 35, there was a significant change in *Lactobacilli* between the 30 and 45 g of NUTRIOSE®FB treatment, but not versus the reference treatment.

A significant change in *total anaerobes* was found. A decrease in total anaerobes was found for the 30 g of NUTRIOSE®FB-treated group and an increase was present in the highest dose of NUTRIOSE®FB-treated group, resulting in a significant difference between both groups on the Δ's between days 35 and –1. The data were, however, all in the normal range.

The α- and β-glucosidase concentration in the faeces increased after NUTRIOSE®FB treatment (Table 5), suggesting increased enzymatic activity. Also, the amount of branched glucose present in the faeces increased.

The differences were significant for the Glucidex®6-treated group versus both NUTRIOSE®FB-treated groups for β-glucosidase concentration and the amount of branched glucose for the whole study period (day 35 to day –1) as already in the first 3 weeks (day 21 – day -1). For α-glucosidase concentration, the difference was significant from day –1 to day 21 for both NUTRIOSE®FB-treated groups versus Glucidex®6, but the difference found between day 35 and day –1 was only significant for the highest dose of NUTRIOSE®FB-treated subjects versus Glucidex®6. There were no significant differences between the NUTRIOSE®FB treatments in enzymatic activity and in the amount of branched glucose.

The branched glucose concentration further increased for 30 g NUTRIOSE®FB Δ_{35-21} $+11.6 \pm 31.7$ mg/g faeces, whereas for 45 g NUTRIOSE®FB, a decrease was seen of Δ_{35-21} -6.3 ± 28.8 mg/g faeces ($P<0.05$).

Gastrointestinal questionnaires. The GI complaints asked during the last 6 days or the last 24 h were rather similar for the three treatments. The question concerning 'stomach rumbling' showed a significant difference between treatments. A decrease in rumbling frequency for the Glucidex®6 treatment of 1.1 ± 1.2 at day -1 to 0.5 ± 0.9 at day 21 was present (Δ_{21-1} -0.6), compared with an increase for the 45 g NUTRIOSE®FB treatment: at day -1, rumbling occurred 0.6 ± 0.7 times a day and at day 21, 1.1 ± 1.3 times a day during the last 24 h (Δ_{21-1} 0.5) ($P=0.016$). No effect was seen with the 30 g NUTRIOSE®FB treatment. 'Rumbling in the stomach' at day 35 was lower than at day 21 for the highest NUTRIOSE®FB treatment. Furthermore, the severity of the stomach rumbling during the last 6 days showed a tendency towards a difference between treatments ($P=0.0570$), with the largest difference between the NUTRIOSE®FB groups.

The categorical part of the questionnaire in which all subjects scored frequency of occurrence of all GI variables as well as food preferences items was similar for all three groups. No significant differences were found in GI complaints or food preferences owing to treatment.

Anthropometry. With respect to the anthropometric data obtained, a trend towards a better weight maintenance with NUTRIOSE®FB supplementation was present. The baseline BW (see Table 1) was increased with Glucidex®6 Δ_{35-1} $+0.8 \pm 1.0$ kg, whereas both NUTRIOSE®FB-treated groups showed similar weights Δ_{35-1} $+0.0 \pm 1.0$ kg ($P=0.07$) as seen at baseline. For body composition (fat mass and fat-free mass) and waist/hip ratio, no differences between treatments over time were seen.

Hydrogen excretion. Fermentation of NUTRIOSE®FB was examined using the hydrogen excretion breath test, until three-quarters of the daily dose of the treatment was consumed. The increase in area under the 8 h curve (p.p.m.*min) was about fivefold for the NUTRIOSE®FB-treated groups versus rather constant for the Glucidex®6-treated group (see Figure 1). This was already seen after 21 days of treatment ($P<0.05$) and remained at this level at 35 days of treatment ($P<0.05$). There was, however, no difference in the level of fermentation due to the dosage of NUTRIOSE®FB supplied.

Energy intake and hunger and satiety feelings. No clear differences in reported energy intake and macronutrient composition of the diet were found for the three groups when intake of day 35 was compared with day -1 (see

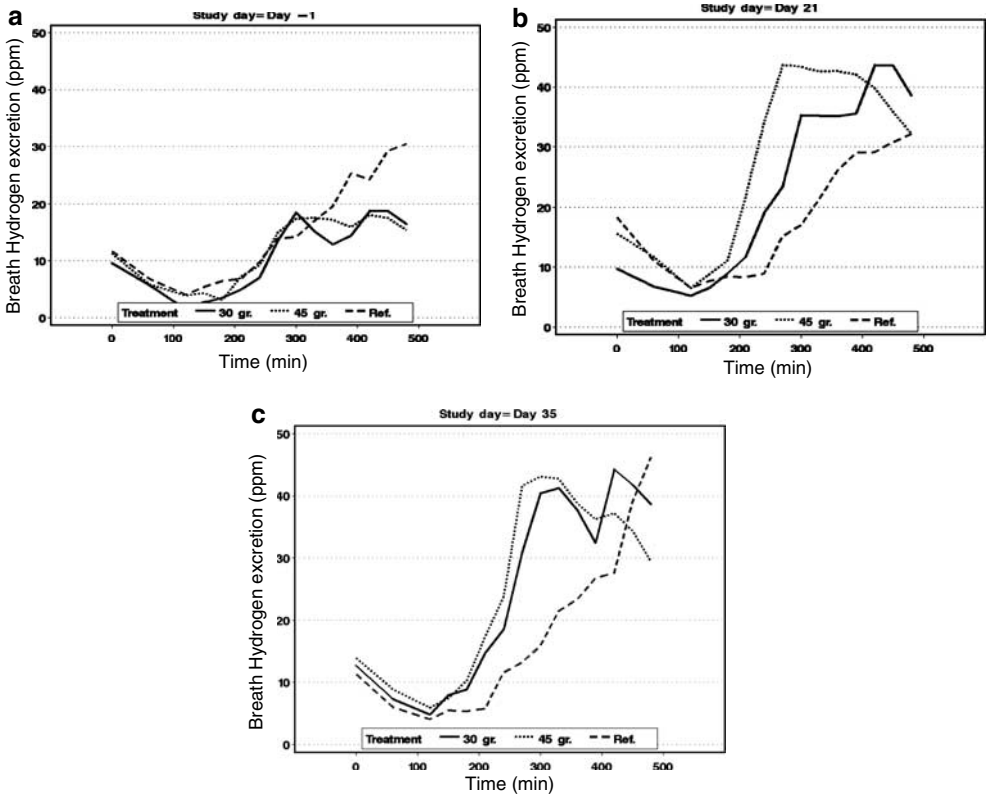

Figure 1 Hydrogen excretion data for the 3 test days (days −1, 21 and 35). The breath test hydrogen excretion is expressed as ppm (*y* axis) versus time (in minutes) (*x* axis).

Table 6 Mean (bold) food intake at the first and last day of the study (standard deviations between brackets)

Treatment	*Glucidex®6 (n = 13)*		*30 g of NUTRIOSE®FB (n = 14)*		*45 g of NUTRIOSE®FB (n = 16)*	
	Day −1	Day 35	Day −1	Day 35	Day −1	Day 35
Energy intake (MJ)	**13.5** (3.7)	**13.3** (3.4)	**12.1** (3.9)	**10.9** (2.1)	**10.8** (3.2)	**10.7** (3.0)
En% protein	**15.9** (1.9)	**16.4** (2.4)	**16.4** (2.5)	**16.8** (2.5)	**15.8** (2.5)	**15.8** (2.0)
En% fat	**32.6** (4.9)	**32.6** (4.1)	**34.7** (5.5)	**34.1** (4.9)	**35.1** (5.4)	**35.6** (5.7)
En% carbohydrate	**48.6** (5.0)	**48.3** (4.3)	**45.7** (6.6)	**45.5** (7.1)	**45.9** (5.3)	**45.9** (5.8)
Dietary fibre(g/MJ)	**2.5** (0.6)	**2.4** (0.5)	**2.7** (0.5)	**2.6** (0.6)	**2.4** (0.6)	**2.4** (0.7)
Dietary fibre and NUTRIOSE®FB (g/MJ)	**2.5** (0.6)	**2.4**[a,b] (0.5)	**2.7** (0.5)	**5.0**[c] (0.8)	**2.4** (0.6)	**6.4** (1.7)
En% alcohol	**2.9** (2.2)	**2.7** (2.2)	**3.1** (2.5)	**3.7**[c] (3.2)	**3.2** (2.2)	**2.7** (2.3)

[a]The change over time between day 35 and day −1 differed significantly between Glucidex®6 and 30 g of NUTRIOSE®FB.
[b]The change over time between day 35 and day −1 differed significantly between Glucidex®6 and 45 g of NUTRIOSE®FB.
[c]The change over time between day 35 and day −1 differed significantly between 30 and 45 g of NUTRIOSE®FB.

Table 6). Hunger and satiety patterns or food preferences during the test days with treatment were similar.

Discussion

In the present long-term study in which 30 and 45 g doses of NUTRIOSE®FB were used, compliance of intake of test products was very high for all treatments (compliance >99.6%). The occurrence of related adverse events was low (15 cases: 13%), with no study-treatment-related dropouts, although more adverse events occurred with the highest dose of NUTRIOSE®FB.

Tolerance

In a previous short-term study, it appeared that 1 week supplementation of different doses of NUTRIOSE®FB, the 30 and 45 g dose, were well tolerated (van den Heuvel *et al*., 2004). With the doses of NUTRIOSE®FB used in the present study, 30–45 g daily, no severe GI complaints were reported. Only the complaint 'rumbling in the stomach' in the last

24 h was more frequently reported on the highest dose of NUTRIOSE®FB compared with reference at the beginning of the study. At the end of the study, there was no significant difference in GI complaints for the different treatments. This probably indicates that after 4–5 weeks consumption, habituation to the dose of NUTRIOSE®FB had occurred.

The low incidence of GI complaints as such for all treatments indicates very clearly the tolerability of NUTRIOSE®FB with doses of 30 and 45 g daily.

Recently, Flood *et al*. reviewed the clinical toleration of polydextrose (PD) in food. It was concluded that these non-digestible 1 kcal/g PD had a mean laxative threshold of about 90 g/day or 50 g for a single dose. The NUTRIOSE®FB supplement showed no laxative problems whatsoever in the present study, with the 30 and 45 g/day dosages tested for 4–5 weeks. No laxative effect of the supplement was present; defeacation frequency and consistency was rather similar for all three treatments as well as wet weight of the collected faeces. In previous experiments also, doses of up to 80 g showed no laxative effect (van den Heuvel *et al*., 2004). With respect to digestive tolerance, NUTRIOSE®FB seems therefore even better tolerated than PD. Therefore, we now established that doses of 30 and 45 g of NUTRIOSE®FB are very well tolerated for a period of 4–5 weeks as well.

Fermentation rate

The results of H_2 breath test, faecal enzymatic activity and the amount of branched glucose present in the faeces clearly illustrate more fermentation on NUTRIOSE®FB treatment compared to Glucidex®6, which is known to be digested completely in the small intestine.

The amount of faecal enzymes present and the amount of branched glucose present are assumed to be a measure for enzymatic activity. The α- and β-glucosidase concentration and the amount of branched glucose present in the faeces increased as expected (see Table 5). The hydrogen breath test results (see Figure 1) also revealed a clear increase in fermentation with NUTRIOSE®FB treatment. For both NUTRIOSE®FB-treated groups, about 60 mg of residual NUTRIOSE®FB (or branched glucose)/g faeces was measured at the end of the study. From the branched glucose data it seems clear that degradation of the branched maltodextrin NUTRIOSE®FB is not further increased when consuming higher dosages of NUTRIOSE®FB. Based on an averaged dry weight data of 20% of an on average spot sample of 70 G in this study, about 840 mg of branched glucose was still present in the faeces. With the highest dose of NUTRIOSE®FB treatment approximately 2% of branched glucose is found in the faeces, and for the lowest dose (with a similar absolute amount), a higher percentage of branched glucose was found (approximately 3%). Furthermore, there seems to be an optimal level that remains stable in the long-term. The highest dose of NUTRIOSE®FB resulted in a faster adaptation of glucose breakdown, resulting in a significant difference between both NUTRIOSE®FB treatments. The group treated with 30 g of NUTRIOSE®FB per day still showed an increase in branched glucose at day 35, while the group treated with highest dose of NUTRIOSE®FB was already maximal at day 21.

In summary, about 97% of NUTRIOSE®FB is digested and fermented, based on faecal analysis. This agrees with findings from the previous study, in which the fermentation was based on the H_2-excretion test (van den Heuvel *et al*., 2004).

Intestinal environment

Although for both doses (30 and 45 g), 97% or more of the added NUTRIOSE®FB is fermented, this does not result in an increase of total SCFA or lactate in the faeces. It is well known that the amount and ratio of SCFA in faecal material does not reflect the production of these acids in the large intestine, since the acids are rapidly absorbed by the epithelium. To truly investigate the production of these acids (and in particular butyrate, which is considered to be a health-promoting metabolite (Roediger, 1991)), fermentation of NUTRIOSE®FB could be tested in *in vitro* experiments, such as in TNO's *in vitro* model of the large intestine (Venema *et al*., 2000).

The pH of the intestines normally varies between 6.5 and 7.0. Total lactate, pH and SCFA were measured as markers for the degree of acidity of the intestinal environment. Supplementation of dietary fibres is thought to lower pH owing to total lactic acid increase and SCFA production. From the data shown, it is, however, not clear which factor caused this lower pH. The total lactate concentration, especially for the 45 g of NUTRIOSE®FB daily, decreased instead of being increased. Also, the amount of SCFA was not different between the treatments. It is possible that fewer bases were present (which were not measured), or that less bicarbonate was secreted by the epithelial cells, both resulting in a decrease in pH.

The decrease in pH of faecal material seems to indicate that NUTRIOSE®FB is fermented relatively slowly, such that the fibre is also fermented in the transversal and distal colon. Saccharolytic fermentation (as indicated by increased glucosidase activity) in these areas of the large intestine precludes proteolytic fermentation with its concomitant production of toxic metabolites (such as ammonia, phenolic compounds, etc.). As these toxic metabolites are assumed to play a role in the development of cancer and inflammation at these sites, this effect of NUTRIOSE®FB can be interpreted as beneficial.

With respect to faecal microbiology, no clear changes were seen as a consequence to treatment. Some bacteria showed an increase in number at the beginning of the treatment, which lowered towards the end, suggesting that other bacteria took over the activity or that adaptation to the dosage of NUTRIOSE®FB supplied took place.

The decrease in pH is also known to affect the number of pathogenic bacteria, resulting in a healthier intestinal environment. Taken into account the definition

of a prebiotic, a microbial food supplement that beneficially affect the host by improving its intestinal microbial balance (Gibson and Roberfroid, 1995), the results found in the present study seem to indicate that NUTRIOSE®FB supplement has some prebiotic characteristics (increased *Lactobacilli*) and affects related side effects (reduction of pH in feacal material, increased glucosidase enzyme content).

Food habits, feelings, intake and resulting body weight

In the present study, 4–5 weeks of NUTRIOSE®FB treatment did not significantly affect food habits or lower energy intake. Satiety and hunger feelings, as well as the 'desire to eat something sweet or savoury' were not at all affected by treatment. Subjective scores as well as objective measures like FFQs showed that NUTRIOSE®FB had no effect at food liking and preferences as well as on actual intake.

In a number of dietary fibre studies, an effect of fibre on energy intake and reduction of hunger and increase of satiety was found (Pasman, 1998). However, in normal weight male subjects, it has been found before that fibre supplementation was not effective (Porikos and Hagamen, 1986; Delargy *et al*., 1995). In obese, and especially in female subjects, restrained subjects food intake decreased with increasing fibre consumption (Pasman *et al*., 1997). The current findings do not preclude that NUTRIOSE®FB may still have a satiating effect in this type of subjects, following an energy-restricted diet as was found by Birketvedt *et al*. (2000). They showed that subject on an energy-restricted diet combined with dietary fibre supplementation had a better BW maintenance after an energy-restricted diet.

In the present study, with respect to body weight, an interesting trend was found. Body weight in both NUTRIOSE®FB-treated groups remained stable, whereas the reference-treated group showed a small increase in body weight. Although no significant difference was found, the trend ($P = 0.07$) is a promising result because of the short period of time (5 weeks) in which this difference was seen and therefore of interest for further study.

In conclusion, daily intake of 30 or 45 g of NUTRIOSE®FB was well tolerated on the long term. No negative effects were found on GI parameters. The product was well fermented, which resulted in an increase in *Lactobacilli* and enzyme content of α-glucosidase and β-glucosidase. NUTRIOSE®FB has therefore pre-biotic characteristics.

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