Net energy value of non-starch polysaccharide isolates (sugarbeet fibre and commercial inulin) and their impact on nutrient digestive utilization in healthy human subjects

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The energy value of NSP has been expressed as their metabolizable energy (ME) content. The aim of the present study was to determine whether differences in ME and net energy (NE) contents were similar for insoluble and soluble NSP. Nine healthy young men were offered three diets according to a Latin-square design (3×3) with three repetitions: diet C (control), diet B (control + 50 g sugarbeet fibre/d) and diet I (control + 50 g commercial inulin/d). After a 16 d adaptation period to NSP isolate, food intake was controlled (duplicate meal method) and faeces and urine were collected for 8 d. A period of 60 h was devoted to measurement of energy expenditure (EE) by whole-body indirect calorimetry. NSP-isolate ingestion induced significant increases in the number of defecations and stool weight resulting from increases in water, DM and microbial mass excretion. After deduction of microbial N, differences in faecal N excretion between diets were not significantly different. Urinary N excretion was slightly decreased by sugarbeet fibre or commercial inulin ingestion but the N balances for the diets were not significantly different. Diet energy, N and lipid apparent digestibilities decreased by only 1–2 %. Commercial inulin was entirely fermented and fermentability of sugarbeet fibre averaged 0·886 (SD 0·117). Sugarbeet fibre and commercial inulin ME values averaged 10·7 (SD 1·2) and 13·0 (SD 2·3) kJ/g DM respectively. NSP-isolate ingestion caused significant (sugarbeet) and non-significant (inulin) increases in daily EE. The maintenance NE contents of sugarbeet fibre and inulin averaged 5·0 (SD 5·0) and 11·9 (SD 1·3) kJ/g DM respectively. Differences in maintenance NE contents of NSP isolates were much greater than differences in ME values.

Non-starch polysaccharides: Sugarbeet fibre: Inulin: Indirect calorimetry

In addition to the well-known beneficial effects of NSP ingestion on satiety and regulation of digestive transit (Cummings *et al*. 1978), epidemiological studies have shown increased incidence of pathologies such as cardio-vascular diseases and colon cancer in Western countries, particularly in western Europe (Trowell, 1972; Southgate, 1988), where NSP intake has decreased greatly during the last few decades (Burkitt, 1987). The regulatory effects of volatile fatty acids (VFA), produced by soluble-NSP fermentation, on colonic mucous membrane cellular development have been demonstrated by Breuer *et al.* (1991) among others. These results have highlighted the beneficial effect of NSP in the prevention of colon cancer.

The potential benefit of greater NSP intake has led to several studies in human subjects on the effect of soluble-fibre ingestion on digestive utilization of dietary components. Thus, ingestion of fruit, vegetable and cereal fibre (+57·5 g/d) resulted in significant decreases in apparent digestibilities of energy, crude protein and lipids (Göranzon *et al*. 1983; Wisker *et al*. 1988; Miles, 1992). From these measurements, the energy value of NSP was expressed in terms of metabolizable energy (ME). However, this energy unit does not take into account energy lost as fermentation heat, or differences in efficiency of glucose and VFA energy utilization, or the probable increase in metabolic rate of the large intestine. The net energy (NE) values of NSP for

Abbreviations: EE, energy expenditure; ME, metabolizable energy; MEm, maintenance ME requirement; NE, net energy; VFA, volatile fatty acids.

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man can be estimated from their measured digestibilities and from estimates of the energy lost in their use for synthesis of microbial biomass, H_2 and CH_4, estimates of the energy lost as heat of fermentation and from the efficiency with which the VFA, products of fermentation, may be utilized (Livesey, 1992). However, all these losses, as well as the probable increase in metabolic rate of the large intestine, can be determined by whole-body indirect calorimetry (Van Es *et al.* 1986).

Because of the beneficial effects of NSP in human nutrition and increasing attention paid to them by the food industry, the aims of the present study were to determine (1) the maintenance NE values of partly insoluble (sugarbeet fibre) and soluble (inulin) NSP isolates and their effect on nutrient digestive utilization in healthy human subjects and (2) whether differences in maintenance NE and ME contents were similar. Energy balances were carried out in subjects adapted to NSP-isolate ingestion, by using whole-body indirect calorimetry which requires relatively high levels of NSP-isolate intake. The best ways to incorporate the isolates into food and the highest tolerable doses were defined from the results of a digestive tolerance study (Seguenot, 1990).

Subjects and methods

Subjects

Nine healthy young men, without any medical history of renal, vascular, digestive, endocrine or currently evolving disease, 21·5 (SD 2·5) years of age, and weighing 69·3 (SD 5·0) kg, were enlisted after a normal physical examination. Those who had a BMI higher than 25 kg/m^2 were also excluded. Each subject received a complete explanation of the purpose and procedures of the investigation and signed an informed consent form. The study protocol was approved by the regional Medical Faculty Ethical Committee (CCPPRB no. AU38). During the study, the subjects lived at home and had lunch and dinner at the Human Nutrition Laboratory. Extra food items, such as alcoholic and energy-containing beverages, were not permitted.

Methods

Experimental design. The nine subjects were offered three diets according to a Latin-square design (3×3) with three repetitions: a control diet (22 g of NSP/d; diet C); a sugarbeet diet (diet B) and an inulin diet (diet I) corresponding to diet C +50 g/d sugarbeet fibre or commercial inulin respectively. Each experimental period, lasting for 28 d, comprised 2 d (days 1 and 2) with the control diet, 14 d (days 3–16) with a progressive adaptation to the NSP isolates up to a maximum of 50 g/d; 12 d (days 17–28) with a constant intake of NSP isolates. The balance period covered 8 d (days 21–28) and involved total collection of faeces and urine; the last 2 d (days 27 and 28) were devoted to measurement of energy expenditure (EE) using whole-body calorimetry. Food intake was determined by the duplicate meal method.

Experimental diets. Four daily balanced low-fibre diets (Table 1) were composed by the dietitian of the Human Nutrition Laboratory. They were distributed in rotation to subjects during each balance period. The ME supply to each subject was calculated from the results of a dietary inquiry, and it was adjusted to appetite over 2 d (days 17 and 18) before the first balance period.

Two types of NSP isolate were studied: sugarbeet fibre and chicory inulin (commercial product containing 620 g pure inulin/kg) produced by the Agro-industries, Recherches et Développements (ARD) Society. Their technological properties allowed their incorporation into

Table 1. Diets. The quantities of the different components shown here are indicative of the actual consumed quantities which were weighed exactly for each subject during each dietary period

Day 1		Day 2	
Lunch	Dinner	Lunch	Dinner
Grated carrots 100 g Dressing 10 g Chicken 120 g Pasta 200 g Margarine 10 g Yoghurt 135 g Compote 100 g Biscuits 30 g Bread 120 g	Vegetable soup 200 g Fish 120 g Rice 200 g Margarine 10 g Cheese 30 g Fruit 200 g Bread 120 g	Cucumber 80 g Cream 10 g Beef 120 g Mashed potatoes 250 g Margarine 10 g Fruit yoghurt 120 g Canned fruit 150 g Bread 120 g	Tomato soup 200 g Eggs (2) 100 g Lentils 150 g Blue cheese 120 g Fruit 150 g Orange juice 200 g Bread 120 g
Day 3		**Day 4**	
Lunch	Dinner	Lunch	Dinner
Peeled tomatoes 100 g Dressing 10 g Veal 120 g Carrots 300 g Margarine 10 g Yoghurt 135 g Dessert cream 100 g Bread 120 g	Vegetable soup 200 g Fish 120 g Potatoes 200 g Butter 10 g Cheese 27 g Biscuits 30 g Orange juice 200 g Bread 120 g	Red beets 100 g Dressing 10 g Beef 120 g Rice 200 g Margarine 10 g Cream cheese 120 g Fruit 150 g Bread 120 g	Vegetable soup 200 g Omelette 120 g Courgette gratin 300 g Cheese 30 g Compote 100 g Biscuits 30 g Bread 120 g

diets, particularly in bread (Seguenot, 1990). Each NSP isolate (50 g) was added to the diet each day in two ways: 30 g in bread before kneading and 20 g sprinkled on liquid foods by the volunteers.

Sample treatment. Representative food samples were prepared during the last 10 d of each experimental period. Duplicate meals and bread were homogenized, freeze-dried and analysed separately. Urine was collected in plastic bottles and weighed daily during the last 8 d of each control period. Representative samples (50 ml/l) were pooled in acid-washed plastic bottles. Faeces were collected in plastic pots, homogenized for the 8 d balance period, freeze-dried and stored at −18° until analysis.

Analytical methods. The DM content of dietary and faecal samples was determined after drying at 80° for 48 h. The gross energy content of dietary samples, faeces and urine was analysed using an adiabatic bomb calorimeter (Gallenkamp, London, UK) calibrated with benzoic acid. Total N content of diets, faeces and urine was analysed using the macro-Kjeldahl method. Lipids of foods and faeces were extracted by the Folch technique (Folch *et al*. 1957), and fatty acids were extracted by lipid saponification with 1·8 M-KOH, acidification with 6 M-HCl and extraction by hexane.

The scheme for carbohydrate analysis is presented in Fig. 1. Starch was analysed in samples of duplicate meals, bread and faeces according to the method of Faisant *et al*. (1995) which includes resistant starch. Glucose was determined enzymically (Merckotest 14365; Merck, Darmstadt, Germany). Before analysis of cell-wall carbohydrates starch was removed from duplicate meal and bread samples using the method of Thivend *et al*. (1972) modified to prevent cell-wall-carbohydrate degradation. In brief, 100 ml distilled water was added to 5 g ground freeze-dried sample, boiled for 5 min and cooled. Then 135 ml acetate buffer (0·3 M, pH 5·0), 12 ml amyloglucosidase (*EC* 3.2.1.3; Merck, 75 IU/mg, 24 g/l) and 0·25 ml Termamyl (Novo 120 L; Novo Nordisk, Fontenay-sous-bois, France) were added. Hydrolysis was carried out in a shaking water-bath for 2 h at 60°. After filtering through a sintered crucible (porosity 2) residue 1 was dried at 60°. The latter was defatted by Soxhlet refluxing with toluene–methanol (2:1, v/v) and filtered through a sintered crucible (porosity 2). Hemicellulose and cellulose in this residue 2 were determined according to Jarrige (1961) after sequential acid hydrolysis and filtering through sintered crucibles (porosity 3), and analysed colorimetrically in the hydrolysates for reducing sugars using xylose and glucose as standards (Besle *et al*. 1981). The cell-wall carbohydrate content of samples containing no (sugarbeet fibre) or little (faeces) starch was determined similarly, except for the long initial step of starch removal. Sugarbeet fibre was washed three times with distilled water at 40°. The hemicellulose content of faeces was corrected by deducting the very low glucose content since starch was totally hydrolysed by refluxing with 1 M-H_2SO_4 for 3 h.

Uronic acids were determined in the water extracts and in the filtrates according to the method of Blumenkranz & Asboe-Hansen (1973). Inulin was hydrolysed from inulin powder, inulin bread, duplicate meals and faeces samples with 0·3 M-$HClO_4$ (80°, 1 h; Beutler, 1984) and analysed enzymically for fructose (Boehringer Mannheim 139106; Boehringer Mannheim, Meylan, France). All the results were expressed as polymers, i.e. taking off one molecule of water per monomeric unit.

Faecal bacteria were extracted as follows: freeze-dried faeces samples were suspended in saline (9 g NaCl/l) solution (100 g washed sample/320 ml) and homogenized three times for 1 min using a Waring blender. The homogenate was pummeled for 5 min in a Colworth Stomacher 400 (A. J. Steward & Co. Ltd., London, UK) using a sterile polyethylene bag (180 × 300 mm) and squeezed through seven layers of surgical gauze. Solid residue was rinsed in saline (100 g/100 ml) and squeezed, as described earlier, through surgical gauze at the end of the procedure. The filtrate was centrifuged at 500 ***g*** for 30 min at 4° (Kontron H401: rotor A 6.14; Kontron, Saint-Quentin en Yvelines, France). The supernatant fraction was retained and the solid fraction was submitted to the same treatment as the initial sample. The two supernatant fractions were pooled and centrifuged at 30 000 ***g*** for 45 min at 4° for isolation of bacteria. The latter were freeze-dried and weighed. The faecal bacterial mass was determined from the purine-base content of the extracted bacteria and faeces samples. Purine bases were precipitated with $AgNO_3$ at pH 2 and quantified by spectrophotometry at 260 nm (Zinn & Owens, 1986).

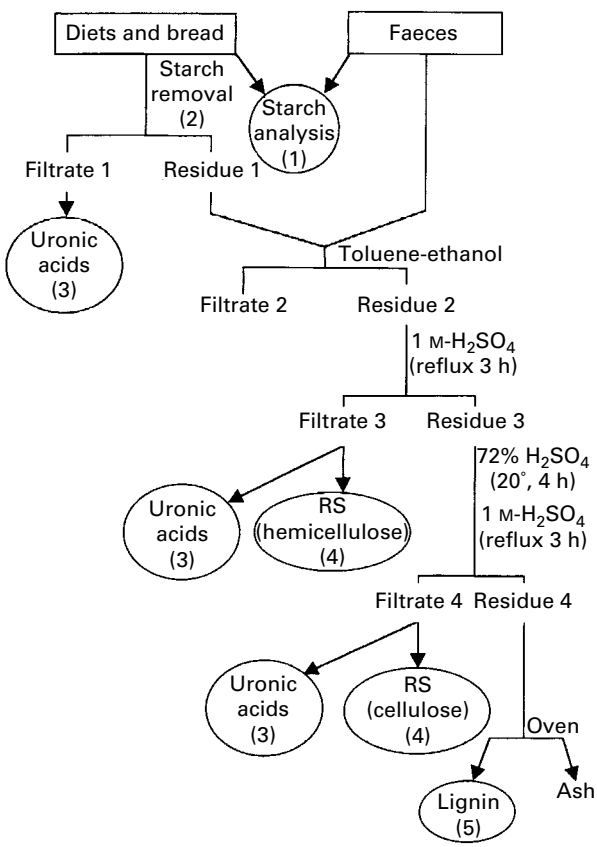

Fig. 1. Flow diagram showing the various stages of carbohydrate analysis. (1), Faisant *et al.* (1995); (2), Thivend *et al.* (1972), modified; (3), Blumenkrantz & Asboe-Hansen (1973); (4), Besle *et al.* (1981); (5), Jarrige (1961). RS, reducing sugars.

Energy expenditure measurements. Whole-body indirect calorimetry was used to determine EE. The two open-circuit calorimetric chambers used (11 m^3 each) were airtight (inflatable seals), continuously ventilated by atmospheric air, and equipped with an air-conditioning system controlling air temperature (± 0·5°) and relative humidity (± 2 %). O_2 consumption and CO_2 production were measured continuously using differential gas analysers: CO_2, 0–1 %; O_2, 21–20 % (Mahiak, Hamburg, Germany). At the end of each balance period, subjects spent 2·5 d in the calorimeters under cardiac supervision, one evening and one night for adaptation to the chamber environment and 2 d for EE measurement. During each measurement period volunteers followed precisely a standardized activity programme with four 20 min periods of exercise on a cycle ergometer (Ergomeca, Sorem, Toulon, France). All physical variables such as air temperature, relative humidity, flow and composition, as well as heart rate were recorded every minute. The validity of gas exchange measurements was checked by infusions of CO_2 and N_2 into the chambers for 8 h after equilibrium (Vermorel *et al.* 1995). Recovery averaged 99·5 (SD 0·6) % for CO_2 and O_2.

Calculations. Computation of nutrient and energy intakes was as previously described (Vernet & Vermorel, 1993). Apparent digestibility of dietary energy was calculated as: ((gross energy intake − gross energy content of faeces)/gross energy intake). Analogous equations were used for N, lipid and fatty acid apparent digestibilities, and NSP (cellulose + hemicellulose + uronic acids) fermentability. Dietary ME was calculated as: digestible energy − urinary energy, because of lack of reliable measurements of H_2 and CH_4 production. Digestible energy values (DEV_{NSP}) and ME values (MEV_{NSP}) of NSP were calculated as follows:

$$DEV_{NSP} = \frac{\left[GEI_{NSP\ diet} \times \left(\frac{DEI}{GEI}\right)_{NSP\ diet}\right] - \ldots}{NSP} \quad \frac{\ldots \left[(GEI_{NSP\ diet} - GEI_{NSP}) \times \left(\frac{DEI}{GEI}\right)_{control\ diet}\right]}{}, \tag{1}$$

$$MEV_{NSP} = \frac{\left[GEI_{NSP\ diet} \times \left(\frac{MEI}{GEI}\right)_{NSP\ diet}\right] - \ldots}{NSP} \quad \frac{\ldots \left[(GEI_{NSP\ diet} - GEI_{NSP}) \times \left(\frac{MEI}{GEI}\right)_{control\ diet}\right]}{}, \tag{2}$$

where DEV_{NSP} and MEV_{NSP} are expressed in kJ/g DM; NSP is expressed as g DM/d; GEI is gross energy intake (kJ/d); DEI is digestible energy intake (kJ/d); and MEI is ME intake (kJ/d).

EE was calculated using the Brouwer (1965) formula (3·866 O_2 (litres) + 1·200 CO_2 (litres) − 1·430 urinary N (g)). Retained energy was calculated as: ME − EE. Maintenance ME requirement (MEm) values for retained energy = 0 were calculated individually from retained energy assuming that ME efficiency was 0·95 for maintenance (negative energy balance) and 0·90 for fattening (positive energy balance) (Van Es *et al.* 1984). Differences in MEm between the experimental diets and the control diet were considered to result from differences in efficiency of NSP-isolate ME utilization for maintenance. The maintenance NE value of NSP (NEV_{NSP}) was calculated as follows:

$$NEV_{NSP} = \frac{ME_{NSP} - \Delta\ MEm}{NSP}, \tag{3}$$

where ME_{NSP} is the ME supplied by NSP (kJ/d). Thus, the NE content of NSP isolate was ME content minus the algebraic difference in MEm between experimental and control diets.

Statistical analysis. Data were analysed statistically according to a Latin-square design (3 × 3) with three repetitions. Comparison between experimental diets was done by ANOVA using the general linear models procedure of Statistical Analysis Systems (1987), according to the following model: $\mu + \alpha$ diet + β repetition + δ subject (repetition) + ε. The 'LS MEAN' statement was used to calculate the adjusted means, and the 'CONTRAST' statement to compare the three diets. For each experimental diet the data are presented as adjusted values, because of lack of one subject on diet I, with standard errors of the mean.

Results

NSP isolate and diet composition

Sugarbeet fibre (15·13 kJ gross energy/g DM) was composed of (g/kg DM): ash 22, crude protein 65, cellulose 233, hemicellulose 272, uronic acids 176, lignin 29, soluble components (proteins, sugars) 203. Commercial inulin (16·65 kJ gross energy/g DM) consisted of (g/kg DM): inulin (fifteen fructose units) 620 and sugars (mono-, di- and trisaccharides) 380. During the cooking of the bread 93 % of the incorporated inulin was hydrolysed. Consequently daily inulin supply was only 22 g instead of 50 g. On average, 0·34, 0·37 and 6·44 g cellulose, 2·22, 2·39, and 9·19 g hemicellulose and 0·17, 0·18 and 4·79 g uronic acids were supplied daily by control, inulin and sugarbeet breads respectively.

All the volunteers completed the study, except one who missed the inulin treatment because of slight diarrhoea. The daily amounts of ingested nutrients and gross energy are given in Table 2. The combined cellulose, hemicellulose and uronic acid intakes were similar for diets C and I but 143 % higher for diet B: 20·0, 19·6 and 48·5 g/d respectively.

Faecal weight and faecal microbial excretion

Daily ingestion of 50 g sugarbeet fibre did not cause digestive disorders in any of the nine volunteers, except a feeling of flatulence in some of them. Ingestion of commercial inulin caused diarrhoea in one subject. However, NSP-isolate ingestion resulted in an increase in the number of

Table 2. Experimentally determined daily nutrient and gross energy intakes of subjects consuming a control diet and diets containing sugarbeet fibre or inulin*

(Mean values and standard deviations for nine subjects per diet)

Diet . . .	Control		Sugarbeet		Inulin	
	Mean	SD	Mean	SD	Mean	SD
Gross energy (kJ/d)	11436	1408	11505	1585	11761	1477
DM (g/d)	560·5	65·5	564·8	68·5	585·7	66·6
Protein (g/d)	113	16	111	15	110	16
(% energy)	23·5		23·0		22·3	
Fat (g/d)	69	15	68	16	64	14
(% energy)	24·5		24·0		22·0	
Total fatty acids (g/d)	54	10	54	14	51	12
Hemicellulose (g/d)	10·4	1·8	21·7	2·5	10·3	1·8
Cellulose (g/d)	4·05	0·82	14·03	2·04	3·93	0·88
Uronic acids (g/d)	5·57	1·24	12·85	1·51	5·38	1·21

* For details of diets, see Table 1 and pp. 344–345.

Table 3. Wet and dry stool weights, faecal bacterial excretion and faecal bacterial nitrogen excretion of subjects consuming a control diet and diets containing sugarbeet fibre or inulin†

(Values are adjusted least square means for nine subjects during an 8 d period, with their pooled standard errors)

Diet . . .	Control	Sugarbeet	Inulin	SEM
Wet faecal weight (g/d)	129^{a}	202^{b}*	204^{b}**	16
Dry faecal weight (g/d)	27·7^{a}	37·6^{b}*	37·1^{b}*	2·3
Faecal bacterial weight (g DM/d)	13·0^{a}	20·3^{b}**	18·6^{b}**	1·2
Faecal bacterial nitrogen (g/d)	0·400^{a}	0·621^{b}**	0·782^{c}***	0·047
Faecal nitrogen (g/d)	1·72^{a}	1·99^{b}*	1·98^{b}*	0·07

^{a,b,c} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
Mean values were significantly different from control: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.
† For details of diet and procedures, see Tables 1 and 2 and pp. 344–346.

defecations (+20 % and +15 % for diets B and I respectively) and a 57 % increase in stool weight ($P < 0·05$) with the two diets (Table 3). The latter resulted from increases in both water and DM excretion ($P < 0·05$). Furthermore, there were large ranges in stool weights (from 59·0 to 281·5 g/d, 62·9 to 393·8 g/d and 71·7 to 297·2 g/d for diets C, B and I respectively) and DM contents, even after correction for differences in food intake whatever the diet.

Daily microbial mass excretion increased by 56 and 43 % with diets B and I respectively ($P < 0·01$, Table 3). The difference in bacterial energy excretion between diets B and C amounted to 35 % of the increase in fermented NSP energy, assuming that the gross energy content of bacteria was 21·3 kJ/g DM as for rumen bacteria (Hussein *et al*. 1995). Furthermore, while the N content of the microbial mass was similar for diets C and B, it was 38 % higher with diet I. Finally, microbial N contributed 23·2, 31·2 and 39·5 % to total faecal N excretion with diets C, B and I respectively.

Apparent digestibility of diets and nitrogen retention

Fermentability of dietary NSP averaged 0·738 (SD 0·087), 0·825 (SD 0·088) and 0·878 (SD 0·056) for diets C, B and I (including inulin) respectively. Hemicellulose and cellulose fermentability was significantly higher for diet B than for diets C and I, whereas uronic acid fermentability was not significantly different between diets B and C (Table 4). However, hemicellulose, cellulose and uronic acid fermentability was significantly lower for diet I than for the other two diets. Variability of cellulose fermentability was high because of very low values for the same two or three volunteers on each of the three diets, resulting in heavier stools and higher stool cellulose content.

Increases in faecal DM excretion with diets B and I were accompanied by significant increases in faecal energy excretion and resulted in significant reductions of apparent digestibility of energy (Table 4). Starch apparent digestibility was over 0·990. Decreases in lipid ($P < 0·01$) and fatty acid (NS) digestibilities were similar with diet B but negligible with diet I. Similarly, the 16 % increase in faecal N excretion ($P < 0·05$) resulted in a 1·8 % reduction of apparent N digestibility with both experimental diets ($P < 0·01$). However, after deduction of microbial N excretion, the corrected N digestibilities obtained were not significantly different between diets C, B and I. Thus, sugarbeet fibre and inulin intake did not significantly alter digestive utilization of dietary proteins and N balance (Table 4).

Table 4. Daily intake, excretion and apparent digestibility values for dietary constituents and fermentability of NSP in subjects consuming a control diet and diets containing sugarbeet fibre or inulin†

(Values are adjusted least square means for nine subjects during an 8 d period, with their pooled standard errors)

Diet...	Control	Sugarbeet	Inulin	SEM
Energy				
intake (kJ/d)	11467[a]	11441[a]	11677[a]	92
faecal excretion (kJ/d)	585[a]	734[b]***	707[b]**	22
apparent digestibility	0·949[a]	0·936[b]**	0·939[b]**	0·002
urinary excretion (kJ/d)	562[a]	543[a]	562[a]	12
Metabolizability	0·900[a]	0·889[b]***	0·891[b]**	0·002
Nitrogen				
intake (g/d)	18·1[a]	17·7[a]	17·6[a]	0·2
faecal excretion (g/d)	1·72[a]	1·99[b]*	1·98[b]*	0·07
apparent digestibility	0·906[a]	0·888[b]**	0·888[b]**	0·004
corrected digestibility ‡	0·927[a]	0·922[a]	0·931[a]	0·003
urinary excretion (g/d)	15·8[a]	15·3[a]	15·3[a]	0·25
balance (g/d)	0·52[a]	0·29[a]	0·29[a]	0·27
Fat				
intake (g/d)	69·2[a]	67·4[ab]	63·3[b]*	1·7
faecal excretion (g/d)	3·72[a]	4·39[b]**	3·52[a]	0·13
apparent digestibility	0·945[a]	0·932[b]**	0·942[a]	0·002
Total fatty acids				
intake (g/d)	54·8[a]	53·4[ab]	50·3[b]*	1·3
faecal excretion (g/d)	1·59[a]	2·00[a]	1·48[a]	0·17
apparent digestibility	0·970[a]	0·959[a]	0·969[a]	0·004
Hemicellulose				
intake (g/d)	10·46[a]	21·50[b]***	10·19[a]	0·37
faecal excretion (g/d)	1·89[a]	2·41[a]	3·16[b]**	0·23
fermentability	0·822[a]	0·881[b]§	0·697[c]***	0·018
Cellulose				
intake (g/d)	4·13[a]	13·86[b]**	3·81[a]	0·34
faecal excretion (g/d)	2·09[a]	4·30[b]§	2·85[a]	0·71
fermentability	0·511[a]	0·687[b]§	0·330[c]§	0·065
Uronic acids				
intake (g/d)	5·61[a]	12·77[b]***	5·37[a]	0·23
faecal excretion (g/d)	0·51[a]	0·77 [ab]	1·08[b]*	0·14
fermentability	0·919[a]	0·939[a]	0·814[b]*	0·026

[a,b,c] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0·05$.
Mean values were significantly different from control: *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$, §$P < 0·1$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 344–346.
‡ Digestibility corrected by subtraction of faecal bacterial N.

Fermentability, digestible energy value and metabolizable energy value of NSP isolates

Fermentability of sugarbeet fibre, calculated from the results obtained with diets B and C, averaged 0·943 (SD 0·066), 0·815 (SD 0·288) and 0·965 (SD 0·035) for hemicellulose, cellulose and uronic acids respectively. As a whole, NSP fermentability averaged 0·886 (SD 0·117) for the nine volunteers and 0·935 (SD 0·056) for seven of them. Only traces of inulin were detected in faeces (from 0 to 3 g/kg DM and less than 1 g/kg, on average) with diet I, so that inulin fermentability was close to 1·00.

Sugarbeet and inulin digestible energy values averaged 11·1 (SD 1·0) and 13·5 (SD 2·1) kJ/g DM respectively. Apparent energy digestibilities of sugarbeet fibre and inulin calculated as the ratio digestible energy value : gross energy (0·731 (SD 0·070) and 0·814 (SD 0·127)) were lower than NSP fermentability.

Urinary energy losses averaged 4·85 (SD 0·43) % of gross energy intake and were not significantly different between the three diets. Ignoring H_2 and CH_4 energy losses, ME amounted to 0·900 (SD 0·015) energy intake with diet C, 0·888 (SD 0·015) with diet B ($P < 0·001$) and 0·891 (SD 0·013) with diet I ($P < 0·01$). ME values of NSP isolates were calculated assuming that decreases in dietary ME contents resulted only from experimental NSP-isolate-intake. Sugarbeet fibre and commercial inulin ME values averaged 10·7 (SD 1·2) and 13·0 (SD 2·3) kJ/g DM respectively, and were not significantly different.

Energy expenditure and maintenance energy requirements of the volunteers

EE of volunteers given the control diet averaged 9·84 (SD 0·83) MJ/d. This high SD may partly result from differences in lean body mass, which was not determined in the present study. However, repeatability of EE measurement was good since the difference between the two consecutive days averaged 1·3 (SD 1·1) %. Daily ingestion of 50 g sugarbeet fibre induced significant increases in EE during the

Table 5. Daily energy expenditure (expressed in kJ/d and as a percentage of the control mean) of subjects consuming a control diet and diets containing sugarbeet fibre or inulin†

(Values for 24 h expenditure are adjusted least square means for nine subjects with their pooled standard error)

Diet . . .	Control	Sugarbeet	Inulin	SEM
Energy expenditure (kJ/d)	9842[a]	10042[b]*	9897[ab]	47
Energy expenditure (% control mean)				
daily	100·0	102·0*	100·5	
sleeping	100·0	102·6*	101·6	
postprandial:				
after lunch (3 h)	100·0	102·0	100·0	
after dinner (5 h)	100·0	106·3*	102·0	
exercise	100·0	104·1*	102·0	

[a,b] Mean values within the row not sharing a common superscript letter were significantly different, $P < 0{\cdot}05$.
Mean values were significantly different from control, *$P < 0{\cdot}05$.
† For details of diets and procedures, see Tables 1 and 2 and pp. 344–346.

various activities and over 24 h. Inulin ingestion caused slight but non-significant increases in EE, especially after dinner and during sleep (Table 5). Retained energy was slightly positive with diet B and a little bit more with diets C and I. MEm values of the volunteers were 2·3 % higher ($P < 0{\cdot}05$) and slightly but not significantly higher with diet I than with diet C (Table 6).

Net energy content of NSP isolates for maintenance

The maintenance NE contents of NSP isolates calculated from their ME content and differences in MEm (see p. 346 and Table 6) averaged 5·0 (SD 5·0) and 11·9 (SD 3·3) kJ/g DM for sugarbeet fibre and inulin respectively (Table 7).

Discussion

Digestive utilization of NSP isolates

The two types of NSP studied (sugarbeet fibre and commercial inulin) were selected because of their technological and nutritional assets and their differences in physicochemical properties. Inulin, a fructose polymer, is characterized by high water-solubility, gel-forming capacity and water-binding capacity (Dysseler & Hoffem, 1995). Although inulin cannot be hydrolysed by the endogenous secretions of the human digestive system, due to the specific structure of the D-fructofuranosyl β(1-2) link, in rats 18–26 % of inulin was digested enzymically in the small intestine and the remainder was almost totally fermented in the distal colon by bacterial microflora (Nilsson & Björk, 1988), especially bifidobacteria and bacteroides (Roberfroid, 1993). By contrast, sugarbeet fibre is mainly composed of insoluble compounds (50·5 % cellulose + hemicellulose) which are fermented in the distal colon. It also contains 17·6 % uronic acids, water-soluble compounds fermented in the proximal colon.

Table 6. Daily metabolizable energy (ME) intake, retained energy (RE) and maintenance ME requirement (MEm) of subjects consuming a control diet and diets containing sugarbeet fibre or inulin*

(Values are adjusted least square means for nine subjects with their pooled standard errors)

Diet . . .	Control	Sugarbeet	Inulin	SEM
ME (kJ/d)	10320[a]	10163[a]	10408[a]	78
RE (kJ/d)	478[a]	121[b]	510[a]	102
MEm (kJ/d)	9776[a]	10010[b]	9831[ab]	54

[a,b] Mean values within a row not sharing a common superscript letter were significantly different, $P < 0{\cdot}05$.
* For details of diets and procedures, see Tables 1 and 2 and pp. 344–346.

The average 57 % increase in wet stool weight obtained with sugarbeet fibre and inulin in the present study agreed with the results previously obtained with various NSP (Kelsay *et al.* 1981). It resulted mainly from an increase in water excretion due to the high water-binding capacity of the products (Cherbut, 1989). Furthermore, as inulin incorporated into bread was hydrolysed during cooking and true inulin intake was only 22 g/d, it can be concluded that inulin had a much higher water-binding capacity than sugarbeet fibre. This assumption is supported by results obtained with rats, since gut content was similar in rats fed on diets containing 80 g inulin/kg or 150 g sugarbeet or carrot NSP/kg (Cubizolles & Vermorel, unpublished results).

Interindividual variability in faecal output was high: the CV were 28, 31 and 22 % for energy, lipids and N respectively. Furthermore, the highest and the lowest values were observed for the same volunteers for the three diets, which stresses the advantage of using a Latin-square design in human studies. The variability of fermentability was much greater for cellulose than for hemicellulose, in agreement with the results of Southgate & Durnin (1970). The lowest values for hemicellulose and cellulose fermentability (close to nil) were obtained for two or three subjects exhibiting the lowest energy digestibility, the highest faecal starch excretion and the greatest bacterial mass excretion. These results might indicate a greater flow of undigested starch into the large intestine, which may have altered fermentation processes to the detriment of the cellulolytic activity of the caecal flora. In addition, the lower fermentability values might result from differences in the composition of the intestinal microflora as suggested by Southgate & Durnin (1970).

Daily ingestion of 50 g sugarbeet fibre was followed by significant increases in dietary cellulose and hemicellulose fermentability. These may have resulted from both the high

Table 7. Determination of net energy (NE) content of sugarbeet fibre and inulin

(Values are means and standard deviations for nine (sugarbeet) or eight (inulin) measurements)

	Sugarbeet fibre		Inulin	
	Mean	SD	Mean	SD
Fibre DM intake (g/d)	43·85		48·45	
Fibre ME intake (kJ/d)	471·7	52·4	627·5	113·2
Difference in MEm (kJ/d)*	249·4	228·8	49·2	233·7
NE (kJ/g DM)	5·0	5·0	11·9	3·3

ME, metabolizable energy; MEm, maintenance ME requirement.
* Difference of MEm between control and fibre diet.

fermentability of the present sugarbeet fibre (processing may have altered its structure and physical properties, Ellis *et al.* 1996) and improved microbial degradation of dietary NSP in the large intestine. In fact, ingestion of the same sugarbeet fibre by growing rats caused a drop in caecal pH and increases in caecal VFA content and percentages of acetate and butyrate in the VFA mixture, which could reflect increased cellulolytic activity of the caecal flora (Cubizolles & Vermorel, unpublished results). By contrast, in the present study, daily ingestion of 50 g inulin resulted in significant decreases in dietary hemicellulose, cellulose and uronic acid fermentability. These results could be partly explained by the development of a specific microflora (Roberfroid, 1993) and alteration of fermentation in the large intestine, as shown in growing rats given inulin: a 54 % increase in caecal VFA concentration and a decrease in the percentage of acetate in the VFA mixture (Cubizolles & Vermorel, unpublished results).

Decreases in apparent digestibility of energy, protein and lipids resulting from sugarbeet fibre or inulin intake in the present study were less than those obtained in other studies with similar quantities of cereal, vegetable or fruit NSP (0·01 *v.* 0·03–0·06) (Göranzon *et al.* 1983; Wisker *et al.* 1988; Miles, 1992). These differences could be partly explained by the higher fermentability of processed sugarbeet fibre (and inulin) in the present study compared with that of undamaged NSP supplied by cereals, vegetables and fruit (Southgate & Durnin, 1970): 0·943 *v.* 0·684 for hemicellulose and 0·815 *v.* 0·259 for cellulose.

The average 50 % increase in faecal excretion of bacterial biomass probably resulted from the increase in carbohydrates fermented in the large intestine. It is noteworthy that dry bacterial mass contributed 50 % or more of the dry stool weight. About 35 % of the fermented sugarbeet NSP energy appeared as faecal bacterial energy, which agrees with the accepted figure of 30 % (range 20–40 %; Livesey, 1992). In other respects the increased excretion of faecal microbial N accounted for the decreased apparent N digestibility of NSP-enriched diets. It may have resulted mainly from urea utilization by the bacterial flora and maybe from desquamation of the enlarged intestinal mucosa. It was compensated for by a slight, non-significant, reduction of urinary N excretion and consequently N balances tended to be reduced in subjects on diets B and I, but were not significantly different between treatments. These results agree with those obtained in human subjects consuming high-NSP diets containing fruit and vegetables (Kelsay *et al.* 1978) and in growing rats fed on diets supplemented with purified NSP sources with a wide range of fermentability (Tetens *et al.* 1996). Furthermore, N retention was significantly increased for the same ME intake in growing rats given sugarbeet fibre or inulin supplements (Cubizolles & Vermorel, unpublished results).

Digestible and metabolizable energy content of diets and NSP

CH_4 and H_2 energy losses could not be determined reliably due to the very low concentrations of these gases in air leaving the calorimetric chambers, so they were not taken into account to calculate the dietary ME content. In adult volunteers given 50 g lactitol monohydrate/d in place of 49 g sucrose/d, H_2 production increased by 1·9 litres/d on average, corresponding to 25 kJ/d, i.e. 0·25 % of daily gross energy intake or 3 % of lactitol gross energy (Van Es *et al.* 1986). Consequently, ignoring gas energy losses probably resulted in errors similar to those for gross energy intake.

The average measured ME contents of the control and NSP diets were compared with ME contents predicted from dietary chemical composition using several published predictive equations (Fig. 2). Agreement was satisfactory for the three diets (average differences lower than 2 %) with the Miller & Payne (1959) and Livesey (1991) predictive equations which assume that the ME content of NSP is close to 8·4 kJ/g. In fact, in the present study the ME contents of sugarbeet fibre and inulin were 10·7 and 13·0 kJ/g respectively. However, the Southgate & Durnin (1970) and Miller & Judd (1984) predictive equations assume a strong depressive effect of NSP on food digestibility and resulted in 3·2 % and 9·6 % underestimation of ME content for diets B and I respectively, whereas agreement was satisfactory for diet C with the Southgate & Durnin (1970) equation (Fig. 2).

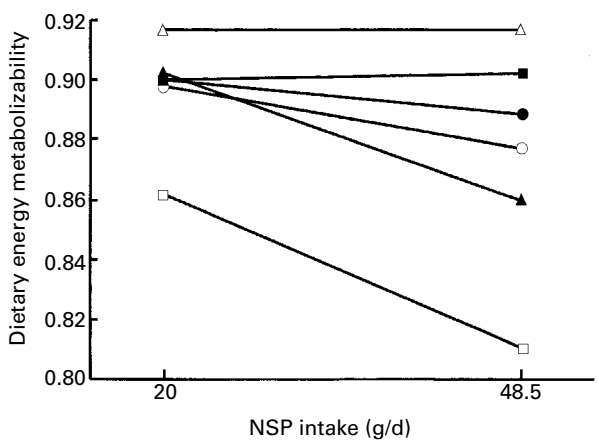

Fig. 2. Variation of dietary energy metabolizability with NSP intake. Comparison of the measured values (●) obtained with control and sugarbeet diets in the present study and those calculated using different predictive equations: (△) Atwater & Bryant (1900); (■) Miller & Payne (1959); (▲) Southgate & Durnin (1970); (□) Miller & Judd (1984); (○) Livesey (1991).

Calculations of digestible energy and ME contents were based on the assumption that increases in faecal energy losses obtained with diets B and I resulted entirely from NSP intake (see p. 346). Faecal energy losses increased on average 3·40 kJ and 2·52 kJ per g sugarbeet fibre or inulin ingested respectively. These results were much lower than those obtained in human subjects with cereal NSP (23·8 kJ/g NSP; Wisker *et al*. 1988).

In the present study the ME content of sugarbeet fibre was similar to that obtained for cereal NSP (Göranzon & Forsum, 1987) and in between those obtained by these authors and Wisker & Feldheim (1990) in human subjects. They agreed with the results obtained in growing rats given the same NSP types at higher feeding levels (Cubizolles & Vermorel, unpublished results). The high fermentability and ME content of inulin were in accordance with the results obtained in rats (Nilsson *et al*. 1988; Cubizolles & Vermorel, unpublished results). However, its ME content was much higher than that (4·18 kJ/g) assumed by Bastiaens *et al*. (1989) and accepted in Belgium and Switzerland (Dysseler & Hoffem, 1995).

Net energy value of NSP isolates

The maintenance NE values of NSP isolates could be calculated from their ME content and the differences in MEm of the volunteers between the experimental and control diets. Such an approach required a high accuracy in the measurement of daily EE, preferably during two consecutive days after adaptation to the facilities, and a Latin-square design to overcome the great interindividual variability of MEm.

The maintenance NE content of inulin in human subjects (11·9 kJ/g DM on average) was close to that obtained in growing rats (10·0 kJ/g DM) using the comparative slaughter method (Cubizolles & Vermorel, unpublished results). It showed a 91·5 % efficiency of ME utilization for maintenance in agreement with the weighted efficiency of VFA (85 %, Krebs, 1960; Armstrong & Blaxter, 1957; Livesey, 1992) and glucose or fructose (95 %, Van Es *et al*. 1986) probably resulting from inulin hydrolysis during bread baking.

The maintenance NE content of sugarbeet fibre (5·0 kJ/g DM on average) was much lower than that of inulin. It resulted from both a lower ME content of sugarbeet fibre and higher MEm of the volunteers. EE was increased during all the considered circadian periods and especially during the postprandial phase. This phenomenon may result from (1) enlargement and thickening of caecal and colonic tissues (Cubizolles & Vermorel, unpublished results) known to have a high metabolic rate, (2) increased motility of the gastrointestinal tract (Cherbut *et al*. 1994) and (3) the lower efficiency of VFA utilization compared with glucose. The great SD of the NE values resulted from the variability of differences in MEm of the volunteers between diets C and B (2·65 (SD 2·53) %). If the two extreme values were excluded, the average NE value of sugarbeet fibre would be 4·4 (SD 3·2) kJ/g DM.

The maintenance NE values of sugarbeet fibre and inulin determined in the present study were compared with those predicted from fibre fermentability, estimated energy lost as microbial mass, H_2, CH_4 and fermentation heat (0·30, 0·02 and 0·05 kJ/kJ carbohydrate fermented respectively) and efficiency of VFA utilization (Livesey, 1992). The predicted NE value of sugarbeet fibre was higher than the measured maintenance NE value (7·6 *v.* 5·0 kJ/g DM) probably because the increased metabolic rate of the digestive tract was not taken into account in Livesey's approach. However, the predicted NE value of inulin agreed with the measured value (12·0 *v.* 11·8 kJ/g DM) probably because the increased metabolic rate of the digestive tract was compensated for by a better efficiency of utilization of fructose, deriving from inulin hydrolysis during bread baking, compared with VFA.

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