

African plant foods rich in non-starch polysaccharides reduce postprandial blood glucose and insulin concentrations in healthy human subjects

Uchenna A. Onyechi, Patricia A. Judd and Peter R. Ellis*

Divisions of Life and Health Sciences, King's College London, Kensington Campus, Campden Hill Road, London W8 7AH, UK

(Received 5 March 1998 – Revised 26 May 1998 – Accepted 1 June 1998)

The effects of two vegetable flours, prepared from the African plants *Detarium senegalense* Gmelin, a legume, and *Cissus rotundifolia*, a shrub, on postprandial blood glucose and insulin concentrations in human subjects, were investigated. Chemical analysis indicated that these flours contained significant amounts of NSP. The detarium in particular was found to be a rich source of water-soluble NSP (SNSP). The flours were incorporated into two types of breakfast meal, a stew meal and a wheat bread meal, containing 50 g and 70 g available carbohydrate respectively. Both meals also contained 10–12 g NSP, the major fraction of which was SNSP. Control and fibre-rich meals were consumed on separate days in randomized order by two different groups of subjects (n 5, stew meals; n 10, bread meals). Venous blood samples were taken at fasting (0 min) and postprandially at 30 min intervals for 2.5 h and the plasma analysed for glucose and insulin. Compared with the controls, detarium and cissus meals elicited significant reductions ($P < 0.006$) in plasma glucose levels at most postprandial time points and for area-under-the-curve (AUC) values (AUC reductions 38–62%). Significant reductions ($P < 0.002$) in plasma insulin levels at various postprandial time points and for AUC values were also seen after detarium and cissus breads (AUC reductions 43 and 36% respectively), but not after the fibre-rich stew meals. SNSP and starch are possibly the main, but not the only, components responsible for the glucose- and insulin-lowering effects of cissus flour. The main SNSP fraction of detarium, identified as a high-molecular-weight xyloglucan, is likely to be a primary factor in determining the physiological activity of detarium flour.

Dietary fibre: Starch: Postprandial carbohydrate metabolism

Water-soluble NSP (SNSP), such as oat β -glucan and guar gum, have received widespread attention as dietary agents that modulate gastrointestinal function as well as lipid and carbohydrate metabolism. Studies in this area are of considerable importance in evaluating the role of SNSP in the aetiology and treatment of diseases, such as diabetes mellitus and cardiovascular disease (Burkitt & Trowell, 1975; Peterson *et al.* 1987; Truswell & Benyen, 1992; Ellis, 1994; Blake *et al.* 1997). Numerous studies have demonstrated that these polysaccharides, when incorporated into starchy foods and glucose drinks, attenuate the postprandial rise in blood glucose and insulin concentrations in healthy and diabetic subjects (Jenkins *et al.* 1976; Ellis *et al.* 1981, 1991; Jarjis *et al.* 1984; Morgan *et al.* 1990; Braaten *et al.* 1992, 1994; Fairchild *et al.* 1996). Animal studies have shown that the postprandial effects of SNSP depend mainly on their capacity to increase the viscosity of digesta in the upper part of the gastrointestinal tract (Cherbut *et al.* 1990; Ellis *et al.* 1995, 1996; Johansen *et al.* 1996). *In vitro* and animal

experiments have indicated that an increase in intraluminal viscosity of digesta is a major factor in inhibiting the rate of digestion and absorption of available carbohydrate (Blackburn *et al.* 1984; Edwards *et al.* 1988; Ellis *et al.* 1995, 1996), although other physico-chemical factors are also involved (Brennan *et al.* 1996).

In rural areas of Nigeria there are numerous plant food preparations used traditionally as thickening agents for soups and stews. These foods increase the viscosity of liquid foods when added as low-moisture flours. This thickening effect could be caused by the presence of starch and/or SNSP. On the basis of these observations, we believed that such plant foods could be a useful source of water-soluble dietary fibre. Preliminary analysis of two of these powdered plant extracts did indeed show that they contained significant amounts of NSP, the major fractions of which were SNSP (Bell *et al.* 1993; Onyechi, 1995). It seemed to us that a detailed investigation of the physico-chemical and nutritional properties of these plant foods was

Abbreviations: AUC, area under the curve; SNSP, water-soluble NSP.

*Corresponding author: Dr Peter R. Ellis, fax +44 (0) 171 333 4082, email p.ellis@kcl.ac.uk

warranted. The two botanical sources selected for testing were those that are traditionally used in Nigeria on a daily basis, namely, *Detarium senegalense* Gmelin, a leguminous plant, and *Cissus rotundifolia*, a shrub. There is a dearth of information in the literature on these two foods, and both appear to be largely uncharacterized and underexploited. One study has shown, however, that these food extracts significantly reduce fasting plasma cholesterol concentrations in rats fed on semi-purified diets containing detarium or cissus flour (Bell *et al.* 1993). As part of our current investigation of the nutritional properties of these materials we have investigated their effects on carbohydrate metabolism in human subjects.

The present investigation was designed to characterize the polysaccharide components of the detarium and cissus flours, using a range of chemical and physical techniques, and determine their effects on postprandial plasma glucose and insulin concentrations in human subjects. The metabolic part of this study involved incorporating these plant food extracts into either a meal of wheat bread and jam, or a meal of rice and mixed stew (containing meat, fish and vegetables). The concept behind using the rice and stew meal was to test the detarium and cissus flours in the way in which they are normally consumed in Nigeria, whilst the wheat bread product was considered to be more acceptable for studies in the UK. We selected healthy, non-diabetic subjects since we were interested in the effects of these plant foods on postprandial glycaemia and insulinaemia in the general population and not just people with diabetes.

Materials and methods

Preparation and processing of plant food extracts

The procedures used in the laboratory for preparing detarium and cissus flour extracts were as close as possible to the traditional methods used by people in rural areas of Nigeria.

Detarium senegalense Gmelin is a leguminous plant belonging to the subdivision Caesalpinoideae (Balogun & Fetuga, 1986) and is considered to be synonymous with *Detarium microcarpum* (Food and Agriculture Organization, 1988). Each pod produced by the plant contains one seed, which is usually rounded, oval or flattened and about 40 mm in diameter (Food and Agriculture Organization, 1988). The legume grows predominantly in West Africa, Chad and Sudan. The seed samples used in the present study were purchased at a local market in Nsukka, Enugu State, Nigeria and then transported to the UK for processing into flour. This was done by boiling the seeds for 45–60 min until the deep brown-purple seed coats (testae) peeled off easily when touched. The testae were then removed and the white cotyledon was soaked in water for 60 min. The cotyledons were washed three times with cold tap water, which was changed each time, and then soaked in water overnight to wash away some of the gummy material. The washed cotyledons were then sun-dried for 24 h and ground into a fine powder to pass through a 1 mm screen (see particle size data on p. 422) with a coffee grinder (Moulinex blender/mill) and air-dried at room temperature for 24 h until the powder did not form lumps when touched.

The powder was yellowish-white in colour with a strong characteristic odour (Onyechi, 1995).

Cissus rotundifolia is a climbing or prostrate shrub found throughout Africa, Egypt and the Arabian Peninsula and is used as a vegetable. It has minor economic importance as a medicinal plant (Balogun & Fetuga, 1986). The stem is sold as a food condiment in local markets in the eastern and northern parts of Nigeria. The cissus flour was produced from the pulverized stem of the cissus plant which was harvested fresh and wet. The bark of the stem was removed by scraping, and the remaining stem cut into small pieces and sun-dried for a week until completely dried. The dried cissus stem was then transported to the UK for further processing, which involved grinding the stem into a fine powder to pass through a 1 mm screen (see particle size data on p. 422) with a coffee grinder (Moulinex blender/mill). The powdered cissus flour was yellowish-brown in colour.

Chemical and physical methods of analysis of plant food extracts

Detarium and cissus flours were analysed using standard methods (Kirk & Sawyer, 1991) for moisture (104° for 16 h), ash (total minerals; 525° for 12 h), fat (Soxhlet; light petroleum-diethyl ether extraction) and protein (Kjeldahl method; $N \times 5.7$). The starch content of the flours was determined by an enzymic method (Englyst *et al.* 1992a). The Englyst method (Englyst *et al.* 1992b) was used to determine total NSP and the water-insoluble fraction of NSP; the water-soluble fraction of NSP was determined as the difference. This procedure involves acid hydrolysis of the NSP followed by gas chromatography of the alditol acetate derivatives of the neutral sugars. The uronic acid content was determined by a sulfuric acid-dimethylphenol colorimetric assay.

The particle size distributions of the detarium and cissus flours were determined by a standard laboratory mechanical sieve method (Lauer, 1966). Purified extracts of the SNSP fractions of the detarium and cissus flours were prepared using a method described in a previous paper (Wang *et al.* 1996). The average molecular weights of the purified SNSP extracts were estimated from measurements of intrinsic viscosity. The molecular weight of detarium xyloglucan can be estimated by calculation of the Mark–Houwink equation using intrinsic viscosity and molecular weight data obtained for tamarind xyloglucan by Gidley *et al.* (1991). Full details of this and the intrinsic viscosity method are reported in our earlier papers (Wang *et al.* 1996, 1997; Blake *et al.* 1997).

Preparation, recipe and composition of experimental meals

Stew meal. The control stew meal was a traditional Nigerian ‘soup’ meal containing meat, fish and vegetables with rice. The following amounts of food ingredients (bought at an African food shop in Peckham, London, UK), expressed as a proportion of the total weight of stew before cooking (g/kg), were used in preparation of the stew meal: 133 chicken breast (without the skin), 66 smoked codfish, 7 dried ground prawns, 66 tomatoes, 53 spinach, 53 onions, 53 red peppers, 20 red palm oil, 8 beef extract (‘Oxo cubes’, Brooke Bond Foods, Crawley, Sussex, UK) and

9 salt. The chicken and smoked fish were cut into small pieces (about 4 cm³) and boiled for 10 min with salt and water. The tomatoes, onions and red peppers, which were homogenized in a blender (Moulinex), and the ground prawns, palm oil and beef extract were added to the stew and then boiled for 10 min. The spinach, which was sliced into pieces of variable size, was also added to the stew and boiled for a further 5 min. A large batch of the stew was frozen and stored at -20° until required. The stews were prepared so that the nutrient composition was the same, except that the two test meals also contained additional NSP from the detarium or cissus flours. Since water-insoluble NSP, as part of an intact plant cell wall, can reduce postprandial glycaemia, probably by acting as a physical barrier to amylase action on starch in the small intestine (Würsch *et al.* 1986), it was decided to prepare meals with similar amounts of NSP. Thus, the detarium meal contained 18 g detarium flour and the cissus stew meal contained 35 g cissus flour, equivalent to 8.2–8.3 g total NSP or 7.7 and 4.9 g SNSP respectively. For presentation to the human subjects on each test day, the vegetable flours were added to thawed stew and mixed well before heating for 10 min in a microwave oven. A batch (225 g) of cooked long-grain rice ('Tilda', Rainham, Essex, UK) was prepared on each test day by adding 600 g cold tap water and boiling for 20 min.

The amount of rice in each meal was adjusted so that the available carbohydrate (mainly starch and sugars) consumed by each subject was 50 g. Meals were served with drinking water such that the weights of the meals were equal (about 600 g). Table 1 provides further details of the amounts and compositions of the stew- and rice-meals, which were calculated from chemical analysis values of the vegetable flours (see p. 422) and from food tables (Holland *et al.* 1991) for the rest of the ingredients.

Bread meals. The bread rolls were prepared using the Chorleywood bread process (Apling & Ellis, 1982) and a simple lean recipe consisting of brown wheat flour ('Ploughman's', Allied Mills, London, UK), salt, yeast

(fresh compressed), fat (hydrogenated vegetable oil; 'Flora', Van den Bergh Foods Ltd., Crawley, Sussex, UK) and 675, 750 and 900 g water/kg flour for the control, cissus and detarium bread recipes respectively. The test flour samples were incorporated into the bread as a replacement for the wheat flour. The weight of the dough piece baked into bread was calculated so that each bread roll contained about 23 g available carbohydrate (mainly starch, sugars). Each detarium and cissus bread roll contained 2.5–2.6 g SNSP. At 2 h after baking, the bread rolls were frozen in self-sealed freezer bags at -20° until required for use. The bread meals consisted of two small bread rolls, 38 g apricot jam ('Robertson's', James Robertson & Sons, Manchester, UK) and sufficient water to make a total meal weight of 400 g.

The total 'available carbohydrate' of each meal was 70 g. All the breads supplied about 46 g available carbohydrate (mainly starch). The apricot jam provided about 24 g available carbohydrate, which consisted of sugars and oligosaccharides (derived from added sucrose, glucose syrups and the apricots) including glucose, fructose, maltose, sucrose, maltotriose, malto-oligosaccharides and maltodextrins. The two test bread rolls provided approximately 5 g SNSP derived from the detarium and cissus flours only. Table 1 provides further details of the amounts and compositions of the bread- and jam-meals, which were calculated from chemical analysis values of the vegetable flours and wheat flour (see p. 422) and from food tables (Holland *et al.* 1991) for the rest of the ingredients. The total sugars content of apricot jam was determined by a refractometric method (Kirk & Sawyer, 1991).

Subjects and ethical approval

Two groups of healthy human subjects, one consisting of five subjects (two males and three females) and the other of ten males, were recruited from staff and student members of King's College London to participate in the stew and bread meals studies respectively. Both acute studies were approved by King's College London Research Ethics Committee.

Table 1. Weights and macronutrient composition of the control meals and meals supplemented with African vegetable flours (detarium and cissus) as consumed by healthy human subjects in stew (*n* 5) and bread (*n* 10) meal studies

	Amount (g)	Protein (g)	Fat (g)	Available carbohydrate* (g)	Total NSP† (g)	Total SNSP† (g)	SNSP from detarium or cissus only (g)	Energy (MJ)
Stew meals:‡								
Control	550	49.7	26.9	49.6	2.7	1.2	0	2.71
Detarium	568	51.4	27.6	49.8	11.0	8.8	7.7	2.77
Cissus	522	49.8	26.8	49.7	10.0	5.7	4.9	2.73
Bread meals:§								
Control	136	10.2	1.9	70.0	5.3	1.8	0	1.48
Detarium	164	11.4	2.6	69.6	10.8	7.1	5.2	1.51
Cissus	148	7.9	1.7	70.1	11.8	6.1	5.0	1.43

SNSP, water-soluble NSP.

* Starch, dextrins, and sugars calculated by difference: 100 - (moisture + protein + fat + ash + total NSP) for the wheat breads and estimated from food tables for the other foods. Starch in cissus flour measured by chemical analysis (Englyst *et al.* 1992a).

† NSP and SNSP derived from all meal ingredients.

‡ Stew meals included different amounts of cooked rice, which were 150, 150 and 87 g for control, detarium and cissus meals, so that each meal contained about 50 g available carbohydrate. Available carbohydrate content of the rice (mostly starch) was 296 g/kg.

§ All bread meals included a 38 g portion of apricot jam. This contained about 24 g available carbohydrate, which consisted mainly of glucose, fructose, sucrose, maltose, maltotriose and maltodextrins. The fructose (as monosaccharide) and sucrose contents were about 26 and 6 g/kg of the total available carbohydrate content of the jam respectively.

Feeding of the subjects

The subjects, who were asked to fast overnight (for at least 12 h), attended our metabolic unit in the Department of Nutrition and Dietetics at King's College London on three separate occasions to consume one control meal and two meals supplemented with the fibre-rich African plant foods (see Table 1). One experimental meal was consumed on each of the visits to the metabolic unit. One group of subjects (n 5) received in random order the control stew meal and the test stew meals containing detarium or cissus flours (pilot study). The other group of subjects (n 10) received in random order a control wheat bread and breads containing detarium or cissus flours (main study). In order to minimize any carry-over effect, the test days were separated by at least 1 week. The subjects were supervised in both studies to ensure that all the meals were consumed within 15 min.

Blood sampling and glucose and insulin assays

On each test day, 10 ml venous blood samples were taken from the subjects at fasting (0 min) and collected in EDTA exetainers. A further five 10 ml blood samples were taken from the subjects postprandially at 30 min intervals for 2.5 h after the meal had commenced. Six samples totalling 60 ml blood were collected from each subject. Blood samples were centrifuged and the plasma decanted and stored at -20° until required for analysis.

Plasma glucose was analysed by a standard glucose oxidase (*EC* 1.1.3.4) method (Werner *et al.* 1970) using the Boehringer Mannheim kit method (GOD-Perid[®] method; Boehringer Mannheim, Lewes, East Sussex, UK) following deproteinization by uranyl acetate solution (URAC[®]). Plasma insulin was measured using the Boehringer Mannheim diagnostic kit method (Enzymun-Test[®] Insulin), which is based on enzyme-immunological reactions for the quantitative determination of human insulin. The ES 22 testing procedure and Combistep 22 analyser program were used for measuring absorbance of a coloured end-point at wavelength 420 nm. The sensitivity of the insulin assay was 0.03 mU/l. Intra-assay and inter-assay CV were 4.3 and 7.3 % respectively.

Statistical analysis

Blood glucose and plasma insulin increments (changes relative to fasting values) were determined at 30, 60, 90, 120 and 150 min. Integrated glucose and insulin increments were estimated by calculation of area under the curve (AUC; trapezoid rule). Glucose and insulin values below the baseline (fasting) were treated as zero. Differences between the effects of the experimental meals on the blood glucose and plasma insulin incremental values were analysed by repeated measures ANOVA with a statistical package (Statistical Analysis Systems, 1985). Significant differences between the control and the test meals were accepted at $P < 0.05$. Post-hoc analysis to examine differences at specific time points was carried out at $P < 0.01$ to compensate for multiple testing.

Results

Chemical and physical characteristics of plant food extracts

Detarium flour. All the results are expressed as g/kg flour sample (dry weight) and indicate that detarium contained 82 fat, 169 protein, 4.0 starch and 27 ash. The total NSP was 638 g/kg of which 598 g/kg was the SNSP fraction and 40 g/kg was the water-insoluble NSP. The main component of the insoluble fraction was found to be cellulose at 23 g/kg sample. The sugar composition of the SNSP fraction indicated high proportions of glucose, xylose, and galactose in the approximate ratio 1.48:1.0:0.55, which is similar to the composition of tamarind seed (*Tamarindus indica* L.) xyloglucan (Reid, 1985). Recent work has confirmed that the SNSP fraction is a xyloglucan (Wang *et al.* 1996). Small amounts of uronic acids were also found, mainly in the SNSP fraction, indicating the presence of small amounts of pectic substances.

The mean particle size of the detarium flour was 464 μ m, with about 95 % of particles < 600 μ m. The intrinsic viscosity of a purified SNSP extract of the detarium flour was found to be about 8.9 SE 0.2 dl/g (Wang *et al.* 1996) and the average molecular weight of detarium xyloglucan was found to be about 2.7 million, allied to a long-chain branched structure (Wang *et al.* 1997).

Cissus flour. All the results are expressed as g/kg flour sample (dry weight) and indicate that cissus contained 9.0 fat, 52 protein, 599 starch and 37 ash. The total NSP, determined by the Englyst method (Englyst *et al.* 1992b), was 263 g/kg, of which 155 g/kg was SNSP and 108 g/kg was water-insoluble NSP. The main component of the insoluble fraction was cellulose (68 g/kg). The sugar composition of the SNSP fraction showed that it contained high proportions of arabinose, uronic acid and galactose in the ratio 1.90:1.0:0.52 respectively. No structural work has been carried out on this fraction which may consist of more than one polysaccharide. The amount of uronic acid suggests however that the SNSP fraction contains a relatively high concentration of pectic substances.

The mean particle size of the cissus flour was 115 μ m with 100 % of particles < 300 μ m. The intrinsic viscosity of a water soluble purified extract of the cissus was found to be about 5.5 (SE 0.4) dl/g, suggesting that the polysaccharide fraction is of relatively high molecular weight, but possibly lower than that estimated for detarium xyloglucan.

Blood glucose and insulin responses for stew meals

The mean age of the healthy subjects (n 5) recruited for the stew study was 30.8 SEM 2.4 years (range 24–38 years) and their mean BMI (weight/height²) was 25.4 (SEM 2.2) kg/m² (range 21.1–32.8 kg/m²).

Glucose. Fasting plasma glucose concentrations of the subjects were found to be within the normal range (World Health Organization, 1985), with a pooled mean of all fasting values of 4.1 (SEM 0.2) mmol/l (range 3.5–4.8 mmol/l). The postprandial rises in plasma glucose concentrations were lower in subjects given detarium and cissus meals compared with the control (Fig. 1(a)). ANOVA of the incremental plasma glucose concentrations showed that there was a significant main effect (Wilks' λ 0.03, F ratio

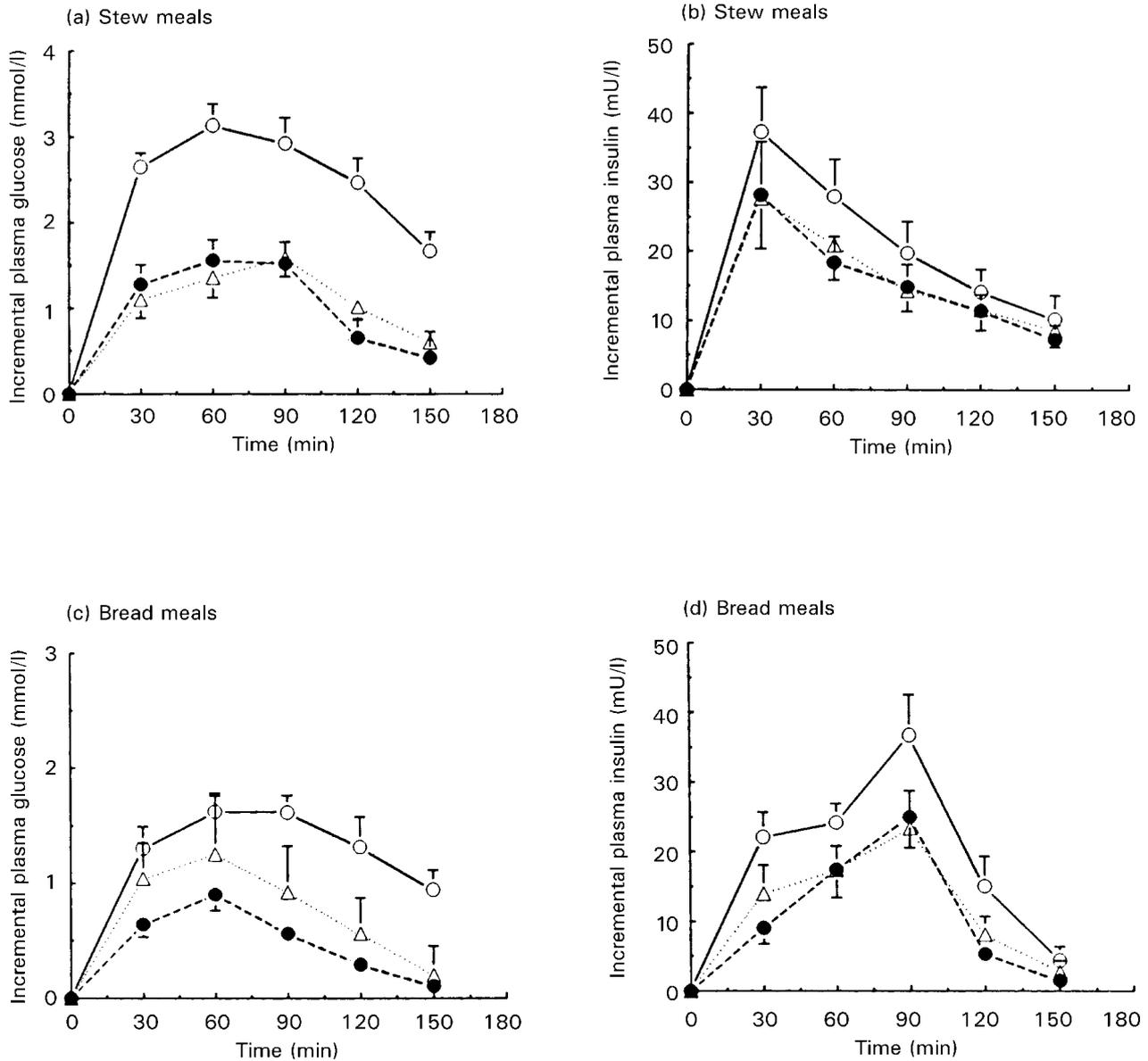


Fig. 1. Incremental plasma glucose and insulin concentrations of healthy human subjects at five time points after meals of stew and rice (a,b) or bread and jam (c,d) containing detarium flour (-●-), cissus flour (···Δ···) or no vegetable flour supplement (-○-). Values are means for five (stew) or ten (bread) subjects, with their standard errors represented by vertical bars. Statistically significant differences between control and test meals at each postprandial time were as follows: (a) glucose: 30 ($P < 0.01$), 60 ($P < 0.001$), 90 ($P < 0.001$), 120 ($P < 0.001$) min for detarium stew meal, and 30 ($P < 0.01$), 60 ($P < 0.001$), 90 ($P < 0.001$), 120 ($P < 0.005$) for cissus stew meal; (b) insulin: no significant differences at any time point; (c) glucose: 60 ($P < 0.01$), 90 ($P < 0.0005$), 120 ($P < 0.005$) and 150 ($P < 0.005$) min for detarium bread meal, and 150 ($P < 0.005$) min for cissus bread meal; (d) insulin: 30 ($P < 0.001$), 90 ($P < 0.0005$) and 120 ($P < 0.01$) min for detarium bread meal, and 90 ($P < 0.01$) min for cissus bread meal.

47.2 on 2 and 3 df, $P = 0.0054$) of the meals. The blood glucose increments at all postprandial times, except 150 min, were significantly lower after the consumption of the detarium and cissus stew meals compared with the control meal (for statistically significant differences at individual time points see legend of Fig. 1). A significant main effect of the meal was also found (Wilks' λ 0.03, F ratio 44.3 on 2 and 3 df, $P = 0.0059$) for the AUC values. Both detarium and cissus meals significantly reduced ($P < 0.001$ and $P < 0.0005$ respectively) the AUC relative to the control (Table 2).

Insulin. Fasting plasma insulin concentrations of the

subjects were within the normal range for healthy adults, the pooled mean of all fasting values was 9.7 (SEM 1.2) mU/l (range 7.1–17.1 mU/l). There was an apparent reduction in the incremental plasma insulin concentration after the consumption of detarium and cissus stew meals compared with the control stew meal (Fig. 1(b)). However, this reduction was not statistically significant (Wilks' λ 0.55, F ratio 1.24 on 2 and 3 df, $P = 0.41$). Also, the detarium and cissus breads did not reduce the AUC compared with the control (Wilks' λ 0.51, F ratio 1.42 on 2 and 3 df, $P = 0.37$; see Table 2).

Table 2. Areas under the curves for postprandial plasma glucose and insulin concentrations (0–150 min) in healthy human subjects consuming stew or wheat-bread meals with or without African vegetable flours (detarium and cissus)

(Mean values with their standard errors for five subjects (stew-and-rice meals) and ten subjects (bread-and-jam meals))

	Glucose (mmol/l.min)		Insulin (mU/l.min)	
	Mean	SE	Mean	SE
Stew meals:				
Control	360	29	3135	423
Detarium	158***	31	2301	502
Cissus	162***	24	2363	548
Bread meals:				
Control	190	21	3003	423
Detarium	73***	9	1723***	307
Cissus	117	37	1930***	401

Mean values were significantly different from those for the control meal:

*** $P < 0.001$.

Blood glucose and insulin responses for bread meals

The mean age of the healthy subjects (n 10) recruited for the bread meal study was 28.7 (SEM 2.3) years (range 21–42 years) and their mean BMI was 24.0 (SEM 1.1) kg/m² (range 18.5–29.4 kg/m²).

Glucose. Fasting plasma glucose concentrations of the healthy subjects were within the normal range (World Health Organization, 1985), with a mean pooled value of 4.3 (SEM 0.12) mmol/l (range 3.8–5.1 mmol/l). ANOVA revealed a significant main effect (Wilks' λ 0.265, F ratio 11.1 on 2 and 8 df, $P = 0.0049$) of the bread meals on the incremental postprandial glucose concentrations (Fig. 1(c)). Comparison of the mean incremental glucose rise after the consumption of bread meals indicated significant differences between control and detarium breads at 60 ($P < 0.01$), 90 ($P < 0.0005$), 120 ($P < 0.005$) and 150 ($P < 0.005$) min, but a significant difference between control and cissus breads was found at 150 ($P < 0.005$) min only. Moreover, when all the time points were compared together, the statistical difference between control and cissus breads was considerably weaker ($P < 0.07$) than that between control and detarium breads ($P < 0.0008$). A significant main effect of the bread meals was found when the integrated values were analysed (Wilks' λ 0.23, F ratio 13.1 on 2 and 8 df, $P = 0.003$). Thus, the AUC for glucose (Table 2) was significantly reduced ($P < 0.0005$) after the detarium compared with the control meal, but this was not seen after the cissus meal ($P < 0.1$). No significant differences were observed between cissus and detarium breads ($P < 0.5$).

Insulin. Fasting plasma insulin concentrations of the healthy subjects (n 10) were within the normal range, with a pooled fasting mean of 15.1 (SEM 1.0) mU/l (range 8.5–21.0 mU/l). ANOVA revealed a significant main effect (Wilks' λ 0.20, F ratio 16.0 on 2 and 8 df, $P = 0.0016$) of the bread meals on the plasma insulin concentrations (Fig. 1(d)). Relative to the control, the detarium bread showed a statistically significant insulin-lowering effect at 30 ($P < 0.001$), 90 ($P < 0.0005$) and 120 ($P < 0.01$) min, whereas the cissus bread produced a significant lowering effect at 90 ($P < 0.01$) min only. The AUC for insulin

(Table 2) was significantly lowered after detarium and cissus compared with the control (Wilks' λ 0.16, F ratio 20.3 on 2 and 8 df, $P = 0.0007$). A significant reduction in the AUC was found after the detarium ($P < 0.0005$) and cissus ($P < 0.005$) bread meals compared with control. No significant difference in the AUC was observed between the detarium and cissus breads ($P < 0.55$).

Palatability of the test bread rolls

No formal palatability evaluation of the bread rolls was carried out. However, subjects (n 10) were questioned at the end of the study about the palatability of the bread. All the subjects considered cissus bread to be of very poor quality even when eaten with apricot jam, indicating that the bread had a poor gritty texture, a poor crust and crumb colour, which was considerably darker brown than the control, and that the flavour was unacceptable. However, the subjects found the sensory qualities of detarium bread to be much more acceptable. The subjects commented that it was difficult to distinguish between the control and detarium breads, a surprising observation in view of the high concentration of detarium SNSP in the product. The authors' evaluation of the breads indicated that it was difficult to detect differences in the texture, flavour and colour between the control and detarium breads.

Discussion

All of the fibre-supplemented stew and bread meals produced substantial reductions in the postprandial rise in plasma glucose concentrations relative to the corresponding controls, with the exception of cissus bread which showed a modest reduction. The glycaemic indices of the detarium stew and bread meals, which were calculated from AUC values (Table 2) using 100 as the reference standard for the control meals, were 44 and 38 % respectively. These values are substantially lower than the glycaemic indices of guar-containing foods seen in studies where subjects consumed SNSP at doses much higher than those used in the current

study (Morgan *et al.* 1990; Ellis *et al.* 1991). Indeed, they are similar to the glycaemic indices of various legumes, which are classified as low-glycaemic-index foods (Jenkins *et al.* 1981; Truswell, 1992).

Significant reductions in the postprandial rise in plasma insulin concentrations were also seen in response to the detarium and cissus bread meals compared with the control. The reductions in the integrated insulin levels (AUC for 0–150 min) after the fibre-supplemented breads were between 36 and 43%, which are of a similar magnitude to those seen in earlier studies using guar-containing bread (Morgan *et al.* 1990; Ellis *et al.* 1991). The insulin-lowering effect of the high-fibre stew meals was not so convincing, however. In fact, although decreases in insulin were seen at all the postprandial times and for integrated values (0–150 min), none of these changes was statistically significant. This can probably be explained by a number of factors including the small number of subjects (n 5) available for statistical analysis and also the large variability in plasma insulin concentrations between subjects (see SE of mean values in Fig. 1 and Table 2).

It seems likely that the SNSP fraction of the detarium seed extract, a high concentration of which is located in highly thickened cell walls of the seed cotyledons (Wang *et al.* 1996), is, at least in part, responsible for the physiological effects of the detarium flour. Chemical analysis has shown that the SNSP fraction, which is about 600 g/kg (dry weight) of the detarium flour, consists mainly of a xyloglucan. This is structurally similar to the main polymer found in tamarind gum, a seed extract of the plant *Tamarindus indica* L. (Reid, 1985), which is also known to have beneficial effects on lipid metabolism in rats (Yamatoya *et al.* 1996). Thus, the detarium xyloglucan consists of a cellulosic backbone with single-unit α -D-xylopyranosyl substituents attached to C-6 of the glucosyl residues, and with some of the xylose residues further substituted at C-2 by β -D-galactopyranosyl residues (Wang *et al.* 1996). Our analysis of detarium xyloglucan shows that it has an intrinsic viscosity of 8.9 dl/g (Wang *et al.* 1996), indicating that it is a high-molecular-weight polymer. This has been confirmed by the results of light-scattering measurements showing that it has a molecular weight of about 2.7 million (Wang *et al.* 1997) and explains why this macromolecule has the capacity to generate high viscosity in aqueous solutions. The xyloglucan fraction seems to be the main component of detarium flour responsible for its viscosity-enhancing effect when added to water or liquid foods, such as the stew meal used in the present study. Rheological studies of semi-dilute (1.0–30 g/kg) solutions of xyloglucan have confirmed that, like tamarind xyloglucan and guar galactomannan, it is a well-behaved linear polymer entanglement network system (Wang *et al.* 1997).

An important determinant of the biological activity of SNSP, such as guar gum and oat β -glucan, is their capacity to generate viscosity in the lumen of the stomach and small intestine. This is now considered to be of primary importance in reducing the rate (and possibly the extent) of digestion and absorption of available carbohydrate (Ellis *et al.* 1996). An increase in the viscosity of stomach contents can impair gastric function, such as sieving and mixing, one consequence of which is an increase in the size of

large-sized food particles entering the small intestine (Meyer & Doty, 1988). Moreover, an increase in digesta viscosity is thought to reduce the rate of emptying from the stomach, although evidence for this is contradictory (Low, 1990; Ellis *et al.* 1996). There is little doubt also that an increase in viscosity in the gut lumen inhibits the propulsive and mixing effects of intestinal contractions (Blackburn *et al.* 1984; Edwards *et al.* 1988). Under these conditions, SNSP are likely to impair the rate of digestion of starch as a result of less disruption of food particles, reduced mixing of food with intestinal secretions and decreased transport of hydrolysed products of starch to the mucosal surface. Some recent evidence also suggests that these polymers, in addition to increasing digesta viscosity, may act as a 'physical barrier' to amylase–starch interactions in the lumen of the small intestine (Brennan *et al.* 1996). Fluorescence microscopy revealed that guar galactomannan was closely associated with starch granules in digesta removed from mid-jejunum of pigs fed with guar–wheat bread (Brennan *et al.* 1996). This may help to explain why detarium elicited a slightly greater glucose-lowering effect in bread than in the stew meal, despite the fact that the bread contained more available carbohydrate and much less xyloglucan than the stew (5.2 and 7.7g SNSP for bread and stew respectively). When preparing the rice-and-stew meal the detarium flour was gently mixed by hand into the stew just before the subjects consumed the meal, so that contact between the xyloglucan and rice starch was minimal before ingestion. In contrast, for the preparation of wheat bread by the Chorleywood bread process, the detarium was thoroughly mixed with the wheat flour and other dough ingredients using a special high-speed mixer (energy input 40 kJ/kg in 2–4 min). Intimate contact between starch and the SNSP of detarium flour is more likely under these mixing conditions.

Based on our knowledge of the physico-chemical properties of the detarium xyloglucan and the results of previous acute studies with other sources of SNSP, the xyloglucan fraction is probably a major factor in determining the biological activity of detarium flour. Other factors cannot be ruled out however; for example, the presence of 'anti-nutritional' factors (Thorne *et al.* 1983), including tannins (Thomson *et al.* 1984), lectins (Rea *et al.* 1985) and phytic acid (Thomson *et al.* 1987) may contribute to the activity of the flour (Thorne *et al.* 1983; Truswell, 1992). However, since the raw detarium bean and the detarium bread (see p. 420) received heat treatment during processing, the effect of some of these 'anti-nutrients' may be trivial if they are inactivated (Giama & Wachuku, 1997).

The explanation of why the cissus-supplemented meals attenuated the postprandial rise in blood glucose and insulin concentrations is rather more complex. Preliminary physical and chemical analyses of the cissus flour indicate that the SNSP fraction is probably partly responsible for its physiological effects. An intrinsic viscosity of 5.5 dl/g was obtained for this fraction, indicating that the molecular weight is possibly lower than that determined for detarium with an intrinsic viscosity of 8.9 dl/g. This is consistent with preliminary data for the solution rheology of the same cissus extract, which produced much lower solution viscosity than the same concentrations of detarium xyloglucan (Onyechi, 1995). From these data, and the fact that the cissus meal

contained nearly 40% less SNSP than the detarium meal, it is difficult to explain why the stew meals containing either cissus or detarium produced similar glucose-lowering effects. One possibility is that water-insoluble NSP, as part of an intact plant cell wall, can reduce postprandial glycaemia by acting as a physical barrier to amylase action on intracellular starch in the gut lumen (Würsch *et al.* 1986). However, preliminary microscopical analysis has shown that the cell walls of the parenchymal tissue of cissus are disrupted during processing and the starch granules are largely detached from this cellular tissue (Y Ren, unpublished results). Another possibility here is that other components in the cissus flour (e.g. amylase inhibitors) impaired the rate and extent of starch digestion. Our results seem to make more sense however when differences in the type of starch between the stew and rice meals containing cissus and detarium are accounted for. About 50% of the total starch in the cissus meal originated from the uncooked cissus *per se*, which was added to the stew part of the meal and then heated in a microwave for 10 min. Under these conditions it is possible that the starch in the cissus flour was less swollen and gelatinized than the starch in the other cooked foods, namely rice and wheat bread. Consequently, the physical state of the cissus starch may partly account for the better-than-predicted glucose-lowering effect of cissus flour in the stew meal. Thus, the starch in the cissus meal may be more resistant to amylase action in the upper gastrointestinal tract (Collings *et al.* 1981; Holm *et al.* 1988; Colonna *et al.* 1992). Some evidence for this comes from the results of rat studies, showing that the digestibility of a semi-purified diet was significantly reduced when it contained cissus flour (Bell *et al.* 1993; Onyechi, 1995). These findings are certainly compatible with the results of our bread meal study. Both fibre-supplemented meals contained similar amounts of SNSP (approximately 5 g), but the cissus bread appeared to be less effective at lowering plasma glucose and insulin concentrations than bread containing detarium. Compared with the cissus stew meal, the heat treatment of cissus bread starch during bread-making may have rendered the starch more easily available for amylolysis *in vivo* thereby increasing the glycaemic response. The results also suggest that the detarium xyloglucan is more effective than the SNSP fraction of cissus. However, to make any definitive statements about this we will need to evaluate the biological activity of purified polysaccharide extracts of both cissus and detarium. Also, it would be useful to obtain detailed information about the anti-nutrients present in flours, and changes in the physico-chemical structure and properties of cissus starch during heat processing.

In conclusion, the results described in this paper have indicated that two previously uncharacterized plant foods indigenous to Nigeria have potential as dietary supplements for improving glycaemic control. As these foods are cheap, easily available and commonly used as food thickeners in rural Nigeria, they could be exploited for the treatment of diabetes in the more urban areas of Nigeria (and other parts of Africa) where the prevalence of diabetes is currently a serious health problem (Johnson, 1991). The detarium in particular shows considerable promise, not just because of its marked glucose- and insulin-reducing effects, but also

because of its apparent lack of deleterious effects on the sensory qualities of wheat bread. Formal sensory studies need to be carried out to confirm this. Detarium and cissus flours are likely to have other interesting nutritional properties that need investigating in human subjects, including its potential effects on lipid metabolism and large-bowel function. An animal study has already shown that these plant materials have hypocholesterolaemic activity (Bell *et al.* 1993). At a more speculative level, it would be interesting to know what prophylactic benefits, if any, these fibre-rich materials have on the development of disease. For example, the prevalence of diabetes, cardiovascular disease and large-bowel cancer is low in rural areas of Africa (Burkitt & Trowell, 1975) where plant foods such as detarium and cissus are important food ingredients.

Acknowledgements

The authors gratefully acknowledge our colleagues at King's College London, including Ms Rosie Calokatsia and Mr David Lincoln for their expert technical assistance, Professor Simon Ross-Murphy for helpful discussions on the physico-chemical properties of polysaccharides, Mr Peter Milligan for advice on statistics and Ms Yukiyo Yamamoto for providing the intrinsic viscosity data on the cissus SNSP fraction. We also thank Dr Hans Englyst (MRC Dunn Clinical Nutrition Centre, Cambridge) for his help with the analysis of the NSP and starch in the African foods, and Mr John Bowery (Technical Coordinator, James Robertson & Sons, Manchester) for providing detailed carbohydrate composition of the apricot jam. We are particularly grateful to all our volunteers for giving their time so freely; this study would not have been possible without their help. U. O. was supported by the Association of Commonwealth Universities.

References

- Apling EC & Ellis PR (1982) Guar bread: concept to application. *Chemistry and Industry* **23**, 950–954.
- Balogun AM & Fetuga BL (1986) Chemical composition of some under-exploited leguminous crop seeds in Nigeria. *Journal of Agricultural and Food Chemistry* **34**, 189–192.
- Bell S, Onyechi UA, Judd PA, Ellis PR & Ross-Murphy SB (1993) An investigation of the effects of two indigenous African foods, *Detarium microcarpum* and *Cissus rotundifolia*, on rat plasma cholesterol levels. *Proceedings of the Nutrition Society* **52**, 372A.
- Blackburn NA, Redfern JS, Jarjis H, Holgate AM, Hanning I, Scarpello JHB, Johnson IT & Read NW (1984) The mechanism of action of guar gum in improving glucose tolerance in man. *Clinical Science* **66**, 329–336.
- Blake DE, Hamblett CJ, Frost PG, Judd PA & Ellis PR (1997) Wheat bread supplemented with depolymerized guar gum reduces plasma cholesterol concentration in hypercholesterolemic human subjects. *American Journal of Clinical Nutrition* **65**, 107–113.
- Braaten JT, Scott FW, Wood PJ, Reidel KD, Poste LM & Collins MW (1994) High β -glucan oat bran and oat gum reduce postprandial blood glucose and insulin in subjects with and without type 2 diabetes. *Diabetic Medicine* **11**, 312–318.
- Braaten JT, Wood PJ, Scott FW, Reidel KD, Poste LM & Collins MW (1992) Oat gum, a soluble fibre which lowers glucose and insulin in normal individuals after an oral glucose load:

- comparison with guar gum. *American Journal of Clinical Nutrition* **53**, 1425–1430.
- Brennan CS, Blake DE, Ellis PR & Schofield JD (1996) Effect of guar galactomannan on wheat bread microstructure and on the *in vitro* and *in vivo* digestibility of starch in bread. *Journal of Cereal Science* **24**, 151–160.
- Burkitt DP & Trowell HC (1975) *Refined Carbohydrate Foods and Disease. Some Implications of Dietary Fibre*. London: Academic Press.
- Cherbut C, Albina E, Champ M, Doublier JL & Lecannu G (1990) Action of guar gum on the viscosity of digestive contents and on gastrointestinal motor function in pigs. *Digestion* **46**, 205–213.
- Collings P, Williams C & MacDonald I (1981) Effects of cooking on serum glucose and insulin responses to starch. *British Medical Journal* **282**, 1032–1033.
- Colonna P, Lelou V & Buléon A (1992) Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition* **46**, Suppl. 2, S17–S32.
- Edwards CA, Johnson IT & Read NW (1988) Do viscous polysaccharides slow absorption by inhibiting diffusion or convection? *European Journal of Clinical Nutrition* **42**, 307–312.
- Ellis PR (1994) Polysaccharide gums: their modulation of carbohydrate and lipid metabolism and role in the treatment of diabetes mellitus. In *Gums and Stabilizers for the Food Industry*, pp. 207–216 [GO Philips, DJ Wedlock and PA Williams, editors]. Oxford: Pergamon.
- Ellis PR, Apling EC, Leeds AR & Bolster NR (1981) Guar bread: acceptability and efficacy combined. Studies on blood glucose, serum insulin and satiety in normal subjects. *British Journal of Nutrition* **46**, 267–276.
- Ellis PR, Dawoud FM & Morris ER (1991) Blood glucose, plasma insulin and sensory responses to guar-containing wheat breads: effects of molecular weight and particle size of guar gum. *British Journal of Nutrition* **66**, 363–379.
- Ellis PR, Rayment P & Wang Q (1996) A physico-chemical perspective of plant polysaccharides in relation to glucose absorption, insulin secretion and the entero-insular axis. *Proceedings of the Nutrition Society* **55**, 881–898.
- Ellis PR, Roberts FG, Low AG & Morgan LM (1995) The effect of high-molecular-weight guar gum on net apparent glucose absorption and net apparent insulin and gastric inhibitory polypeptide production in the growing pig: relationship to rheological changes in jejunal digesta. *British Journal of Nutrition* **74**, 539–556.
- Englyst HN, Kingman SM & Cummings JH (1992a) Classification and measurement of nutritionally important starch fractions. *European Journal of Clinical Nutrition* **46**, Suppl. 2, S33–S50.
- Englyst HN, Quigley ME, Hudson GJ & Cummings JH (1992b) Determination of dietary fibre as non-starch polysaccharides by gas-liquid chromatography. *Analyst* **177**, 1707–1714.
- Fairchild RM, Ellis PR, Byrne AJ, Luzio SD & Mir MA (1996) A new breakfast cereal containing guar gum reduces postprandial plasma glucose and insulin concentrations in normal-weight human subjects. *British Journal of Nutrition* **76**, 63–73.
- Food and Agriculture Organization (1988) *Traditional Food Plants: A Source Book for Promoting the Exploitation and Consumption of Food Plants in Arid, Semi-Arid and Semi-Humid Lands of Eastern Africa*, pp. 243–245. Rome: FAO.
- Giami SY & Wachuku OC (1997) Composition and functional properties of unprocessed and locally processed seeds from three underutilized food sources in Nigeria. *Plant Foods for Human Nutrition* **50**, 27–36.
- Gidley MJ, Lillford PJ, Rowlands DW, Lang P, Dentini M, Crescenzi V, Edwards M, Fanutti C & Reid JSG (1991) Structure and solution properties of tamarind-seed polysaccharide. *Carbohydrate Research* **214**, 299–314.
- Holland B, Welch AA, Unwin I, Buss DH, Paul AA & Southgate DAT (1991) *McCance and Widdowson's The Composition of Foods*, 5th ed. Cambridge: Royal Society of Chemistry/Ministry of Agriculture, Fisheries and Food.
- Holm J, Lundquist I, Björk I, Eliasson AC & Asp N-G (1988) Degree of starch gelatinisation digestion rate of starch *in vitro*, and metabolic responses in rats. *American Journal of Clinical Nutrition* **47**, 1010–1016.
- Jarjis HA, Blackburn NA, Redfern JS & Read NW (1984) The effect of ispaghula (Fybogel and Metamucil) and guar gum on glucose tolerance in man. *British Journal of Nutrition* **51**, 371–378.
- Jenkins DJA, Leeds AR, Gassull MA, Wolever TMS, Goff DV, Alberti KGMM & Hockaday TDR (1976) Unabsorbable carbohydrates and diabetes: decreased post-prandial hyperglycaemia. *Lancet* **2**, 170–174.
- Johansen HN, Bach Knudsen KE, Sandström B & Skjøth F (1996) Effects of varying content of soluble dietary fibre from wheat flour and oat milling fractions on gastric emptying in pigs. *British Journal of Nutrition* **75**, 339–351.
- Johnson TO (1991) Diabetes in the Third World. *World Health* May–June, 8–10.
- Kirk RS & Sawyer R (1991) *Pearson's Chemical Analysis of Foods*, 9th ed. Edinburgh: Churchill Livingstone.
- Lauer O (1966) *Grain Size Measurements on Commercial Powders*. Ausberg: Alpine AG Ausberg.
- Low AG (1990) Nutritional regulation of gastric secretion digestion and emptying. *Nutrition Research Reviews* **3**, 229–252.
- Meyer JH & Doty JE (1988) GI transit and absorption of solid food: multiple effects of guar. *American Journal of Clinical Nutrition* **48**, 267–273.
- Morgan LM, Tredger JA, Wright J & Marks V (1990) The effect of soluble- and insoluble-fibre supplementation on post-prandial glucose tolerance, insulin and gastric inhibitory polypeptide secretion in healthy subjects. *British Journal of Nutrition* **64**, 103–110.
- Onyechi U (1995) Potential role of indigenous Nigerian foods in the treatment of non-insulin dependent diabetes mellitus. PhD Thesis, University of London.
- Peterson DB, Ellis PR, Baylis JM, Fielden P, Ajodhia J, Leeds AR & Jepson EM (1987) Low dose guar in a novel food product: improved metabolic control in non-insulin dependent diabetes. *Diabetic Medicine* **4**, 111–115.
- Rea R, Thompson LU & Jenkins DJA (1985) Lectins in foods and their relation to starch digestibility. *Nutrition Research* **5**, 919–929.
- Reid JSG (1985) Cell wall storage carbohydrates in seeds. Biochemistry of the seed 'gums' and 'hemicelluloses'. *Advances in Botanical Research* **11**, 125–155.
- Statistical Analysis Systems (1985) *SAS® User's Guide: Statistics*, version 5 ed. Cary, NC: SAS Institute Inc.
- Thompson LU, Button CL & Jenkins DJA (1987) Phytic acid and calcium affect the *in vitro* rate of navy bean starch digestion and blood glucose response in humans. *American Journal of Clinical Nutrition* **46**, 467–473.
- Thompson LU, Yoon JH, Jenkins DJA, Wolever TMS & Jenkins AL (1984) Relationship between polyphenol intake and blood glucose response of normal and diabetic individuals. *American Journal of Clinical Nutrition* **39**, 745–751.
- Thorne MJ, Thompson LU & Jenkins DJA (1983) Factors affecting starch digestibility and the glycemic response with special reference to legumes. *American Journal of Clinical Nutrition* **38**, 481–488.
- Truswell AS (1992) Glycaemic index of foods. *European Journal of Clinical Nutrition* **46**, Suppl. 2, S91–S101.
- Truswell AS & Benyen AC (1992) Dietary fibre and plasma lipids: potential for prevention and treatment of hyperlipidaemias. In *Dietary Fibre – A Component of Food. Nutritional Function in Health and Disease*, pp. 295–332 [TF Schweizer and CA Edwards, editors]. London: Springer Verlag.
- Wang Q, Ellis PR, Ross-Murphy SB & Burchard W (1997)

- Solution characteristics of the xyloglucan extracted from *Detarium senegalense* Gmelin. *Carbohydrate Polymers* **33**, 114–124.
- Wang Q, Ellis PR, Ross-Murphy SB & Reid JSG (1996) A new polysaccharide from a traditional Nigerian plant food: *Detarium senegalense* Gmelin. *Carbohydrate Research* **284**, 229–239.
- Werner W, Rey HG & Wielinger H (1970) On the properties of a new chromogen for the determination of glucose in blood according to the GOD/POD methods (In German). *Zeitschrift für Analytische Chemie* **252**, 224–228.
- World Health Organization (1985) *Diabetes Mellitus. Technical Report Series* no. 727. Geneva: WHO.
- Würsch P, Del Vodovo S & Koellreutter B (1986) Cell structure and starch nature as key determinants of the digestion rate of starch in legume. *American Journal of Clinical Nutrition* **43**, 25–29.
- Yamatoya K, Shiakawa M, Kuwano K, Suzuki J & Mitamura T (1996) Effects of hydrolyzed xyloglucan on lipid metabolism in rats. *Food Hydrocolloids* **10**, 369–372.