Postprandial glycaemic, lipaemic and haemostatic responses to ingestion of rapidly and slowly digested starches in healthy young women

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The objective of the present study was to investigate the postprandial metabolism of two starches with contrasting rates of hydrolysis *in vitro*. Characterized using the Englyst method of *in vitro* starch classification, C*Set 06 598 contained predominantly rapidly digestible starch and C*Gel 04 201 contained predominantly slowly digestible starch. Each test starch, naturally enriched with ^{13}C, was fed to ten healthy female volunteers as part of a moderate fat test meal (containing 75 g test starch and 21 g fat), in a double-blind randomized crossover design. The metabolic response to each starch was measured after an overnight fast, in an acute 6 h study, before and after 14 d of daily consumption of 75 g test starch. During each acute study, blood samples were taken at 15 min intervals for the first 2 h and at 30 min intervals for the remaining 4 h. Breath $^{13}CO_2$ enrichment was measured at the same time points and indirect calorimetry was performed for 20 min every 40 min immediately before and throughout the study. Significantly more rapid, greater changes in postprandial plasma glucose, NEFA and serum insulin concentrations were observed after consumption of the rapidly digestible starch. Breath $^{13}CO_2$ output over the first 3–4 h rose rapidly then began to decline following consumption of the rapidly digestible starch, but plateaued for the slowly digestible starch. The 14 d adaptation period did not affect any of the glycaemic or lipaemic variables but there was a reduction in postprandial plasminogen activator inhibitor-1 concentrations. These data confirm that starches characterized as predominantly rapidly digestible versus slowly digestible by the Englyst procedure provoke distinctly different patterns of metabolism postprandially.

Starch digestion: Glycaemia: Insulinaemia: Haemostasis

The joint FAO/WHO (1998) committee recommended consumption of a diet containing at least 55 % of total energy from carbohydrates, to maintain health and prevent disease. Variation in the source of carbohydrate was also advised, in acknowledgement of the diverse metabolic responses to different food carbohydrates. One of the most important metabolic responses is the rate and extent of glucose absorption and subsequent insulin secretion. When a normal concentration of insulin in blood produces a less than normal biological response, the individual is termed 'insulin resistant' (Wallace & Matthews, 2002). This sub-optimal state predisposes individuals to the complex metabolic abnormalities implicated in the aetiology of cancer (Argiles & Lopez-Soriano, 2001; Yoshikawa *et al.* 2001), ageing (Facchini *et al.* 2000), CVD and diabetes (Reaven, 1995). As the amount, rate and extent of carbohydrate digestion are key determinants of postprandial glucose and insulin response (Wolever, 2000), modulation of carbohydrate digestion patterns could provide many health benefits. However, predicting the glycaemic response to a carbohydrate has proved difficult. Unlike dietary fats, which are categorized in food composition tables to identify key

characteristics, e.g. saturated and unsaturated, and family, e.g. *n*-6 and *n*-3, at best food tables provide data on total starch. Glycaemic predictions therefore rely upon a combination of *in vitro* procedures which classify starches according to their rate of hydrolysis (Englyst *et al.* 1992, 1996, 1999, 2000; Champ, 1999), or *in vivo* measurements of glycaemic index (GI) (Jenkins *et al.* 1981, 2002; Bornet *et al.* 1989; Wolever, 2000) and studies using stable isotope-labelled carbohydrates (Normand *et al.* 1992; Vonk *et al.* 2000).

Given the advances in understanding the roles of food starches in health, there is a need to categorize starches according to their rate and extent of small intestinal digestion. Consumption of slowly digestible starches could assist the prevention and management of insulin resistance and diabetes, thus potentially alleviating the increasing burden of the associated non-communicable diseases. The aim of the present study was to investigate two commercially available contrasting waxy maize food starches, naturally enriched with ^{13}C, with differing rates of *in vitro* hydrolysis. The effects of each starch on postprandial glycaemia, insulinaemia, lipaemia and haemostasis were assessed in healthy

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Abbreviations: AOC, area over the curve; AUC, area under the curve; GI, glycaemic index; PAI-1, plasminogen activator inhibitor-1.

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pre-menopausal women. Potential adaptations to starch exposure were also investigated by making these measurements before and after 14 d of daily test starch consumption.

Experimental methods

Subjects

The experimental protocol for the present study was approved by the joint ethics committees of Newcastle and North Tyneside Health Authorities. Each subject gave their informed, written consent to participate. All studies were conducted in the Wellcome Research Laboratories, University of Newcastle, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

Ten healthy pre-menopausal women, aged 20–37 years, were recruited from the student and staff population of the University of Newcastle upon Tyne. All volunteers had a BMI between 20 and 29 kg/m^2 and were not pregnant, lactating, a smoker, or taking medication known to alter insulin sensitivity, carbohydrate or lipid metabolism, and were not following a diet for medical reasons. Fasting blood cholesterol, glucose and insulin concentrations were within the normal range for each participating volunteer.

The experimental starches and test meal

The experimental starches were C*Set 06598, a rapidly digestible pregelatinized thinned waxy maize starch (coded starch A) and C*Gel 04201, a slowly digestible native waxy maize starch (coded starch B) and were provided by Cerestar (Vilvoorde, Belgium). The starches were selected following *in vitro* digestion of a range of commercially available food starches, using the protocol described by Englyst *et al.* (2000), to identify starches with contrasting contents of slowly and rapidly digestible starch. The test starches were supplied pre-weighed, packaged and coded by Cerestar, to ensure 'blinding' of the research team. 'Milkshake' test meals containing 75 g of either test starch (allocated to each volunteer using a random numbers table), 250 g skimmed milk, 40 g double cream, 7 g cocoa powder, 2·6 g artificial sweetener (Tesco own brand) and 100 g chilled water were prepared immediately before consumption by the volunteers. This provided a palatable starch meal with a moderate fat content of 21 g known to represent a standard meal, without flattening the postprandial glycaemic response (based on work by Normand *et al.* 2001).

The experimental protocol

Each volunteer participated in two experimental periods, in a randomized, double-blind, crossover design. During each experimental period, volunteers attended two acute studies. The first study examined the effect of test starch consumption against a background of the subjects' habitual diet. The second, identical acute study was carried out at the end of a 14 d 'adaptation' period, during which the volunteers were asked to replace regularly consumed dietary starch with 75 g of the test starch on a daily basis. All volunteers received advice and guidance on the most appropriate methods of incorporating the test starches into their diets. The first experimental period was followed by a 2-week 'washout' period, before the second experimental period with the second test starch was carried out. This allowed 1 month to elapse between the beginning of the first and second experimental periods, thus allowing the investigation of responses to each starch to occur as close as possible to a fixed point in the volunteers' menstrual cycles. The experimental protocol is summarized in Fig. 1.

For the 24 h prior to each acute study day, each volunteer was advised to avoid alcohol, strenuous exercise and ^{13}C-containing foods. A standard ^{13}C-free evening meal was provided and consumed before 21.00 hours after which the volunteers fasted overnight. This meal consisted of 'Three cheese and broccoli bake' (450 g), baked potato (180 g), lettuce (150 g), strawberries (100 g) and grapes (100 g). For each acute study day, subjects were admitted at 08.00 hours and an intravenous cannula was inserted into the antecubital vein for collection of baseline fasting and postprandial blood samples. The test meal was consumed within 10 min and postprandial blood and breath samples were collected every 15 min for the first 2 h and every 30 min for the remaining 4 h. Indirect calorimetry measurements were made using a Deltatrac Indirect Calorimeter (Datex Instrumentarium Corporation, Helsinki, Finland) for 20 min at 40 min intervals, from 20 min before consumption of the test meal to the end of the study. Throughout the study the volunteers rested in bed and were allowed unlimited access to drinking water.

Habitual and test starch dietary intake of subjects and anthropometric measurements

The volunteers' food intake was estimated from 7 d food records collected before the study commenced, to assess baseline (habitual) food intake, and during each period of test starch 'adaptation', to
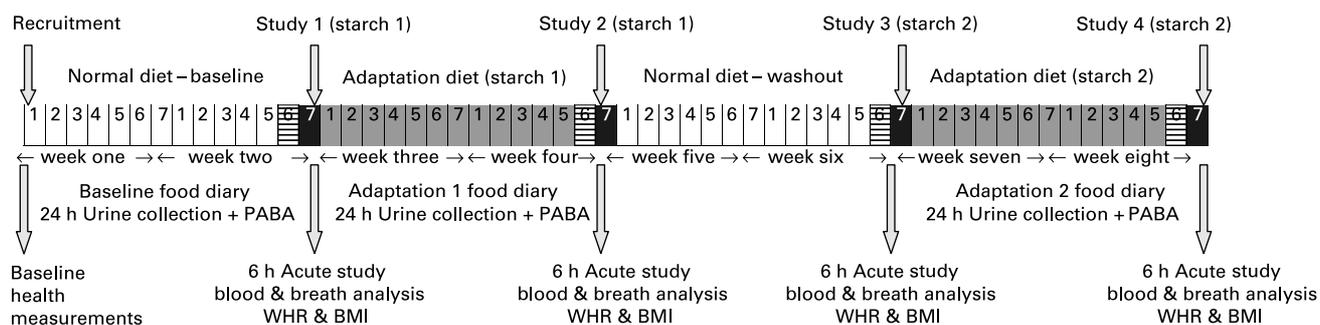

Fig. 1. Experimental protocol summary. ▤ In the 24 h prior to each study, strenuous activity, alcohol consumption and ^{13}C-rich foods were avoided; the evening prior to each study the standardized meal was consumed and each volunteer was asked to fast from 9.00 hours (still water available *ad libitum*). PABA, *p*-aminobenzoic acid; WHR, waist:hip ratio.

determine to what extent the habitual diet was altered by the supplemental test starch. Food portion sizes were quantified using the aid of a photographic atlas (Nelson *et al.* 1997) and face-to-face interviews with the subjects upon completion of the record. Nutrient intakes were calculated from food records using WinDiets Nutritional Analysis Software Suite (Robert Gordon University, Aberdeen). The food records were validated by comparing estimated food N intake with 24 h urinary N excretion. Completeness of the urine collection was assessed through recovery of a 240 mg oral dose of *p*-amino-benzoic acid in urine (Bingham and Cummings, 1983, 1985). Weight, height and waist–hip circumference measurements were taken at the start of each acute study day for each volunteer.

Analytical methods

Plasma glucose, NEFA and serum insulin concentrations were determined using standard enzymic procedures as described previously (Daly *et al.* 1998, 2000). The fasting and 6 h postprandial blood haemostasis markers were assayed as follows: (1) plasma plasminogen activator inhibitor-1 (PAI-1) was analysed using an ELISA kit from Technoclone (Dorking, surrey; Art.-Nr.12 075); (2) plasma fibrinogen analysis was carried out on an automated coagulation analyzer (COAG-A-MATE MTX II; Biomerieux, Durham NC, USA) using MDA Fibriquick (Biomerieux). Whole blood cholesterol was determined using an enzymatic hydrolysis and oxidation performed on an Olympus automated chemistry analyser (Middlesex, UK; model AU640). Enrichment of $^{13}CO_2$ in breath and enrichment of ^{13}C in the test starches were determined by isotope ratio MS using an ANCA 20:20 mass spectrometer (Europa Scientific, Crewe, UK).

Calculations and statistical analyses

Area under the curve (AUC) and area over the curve (AOC) were calculated using the standard trapezoid procedure. The rate of excretion of ^{13}C as $^{13}CO_2$ in breath was calculated as the product of $^{13}CO_2$ enrichment (percentage of atoms above background) and CO_2 production (determined by indirect calorimetry at each time-point during the experimental period) as described previously (Daly *et al.* 2000; Seal *et al.* 2003). The cumulative output of ^{13}C over the 360 min experimental period was calculated from the incremental AUC for $^{13}CO_2$ output and expressed as a percentage of the original ^{13}C dose above background for each individual.

All data were analysed statistically using the ANOVA general linear model and MINITAB (Coventry, UK) release 13. Of the ten volunteers recruited, all ten successfully completed both study days for starch B, but due to cannulation problems, one of the volunteers was unable to complete the studies for starch A.

Results

In vitro starch hydrolysis

The release of glucose following hydrolysis by the Englyst *et al.* (2000), method of starch classification for the two test starches, is illustrated in Fig. 2. Starch A contained 95·5 % rapidly digestible starch and 0·0 % slowly digestible starch, compared with starch B, which contained only 46·8 % rapidly digestible starch and 45·5 % slowly digestible starch. As a result starch A was rapidly
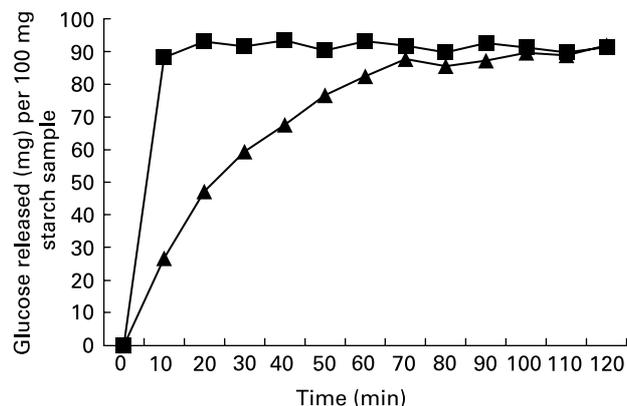

Fig. 2. Cumulative appearance of glucose during *in vitro* hydrolysis of the test starches (■ C*Set 06598 coded starch A; ▲ C*Gel 04 201 coded starch B) selected for *in vivo* analysis. The digestion conditions were those described by Englyst *et al.* (2000).

hydrolysed, with glucose release reaching a plateau quickly after just 10 min, compared with starch B, which was slowly hydrolysed, reaching a plateau after 70 min. The resistant starch content for both starches was low, at 5·2 % for starch A and 7·7 % for starch B.

Subject characterization

Each volunteer recruited was young, relatively lean and healthy (Table 1). Habitual daily energy and nutrient intakes (Table 2) show that, on average, 30 % of dietary energy was derived from fat, which more than meets the Department of Health (1991) recommendations. In addition, carbohydrates provided on average approximately 50 % of dietary energy. Whilst these values differed significantly ($P < 0·05$) between individual volunteers, no variation occurred between the three periods of dietary recording. Starch intake as a percentage of total energy was significantly higher during both 14 d periods of test starch consumption, when compared with baseline. Starch intake as a percentage of total carbohydrate was greater during the 14 d periods of test starch consumption, but this was significant only for test starch B.

Nutrient intake data were verified by the comparison of urinary N output with N intakes from analysis of the food diaries, which showed a mean ratio of 0·84 across all three periods. The completeness of the 24 h urine collections were verified by *p*-amino-benzoic acid recoveries which averaged 98·9 % (Table 2).

Test starch adaptation period (14 d)

With the exception of the postprandial PAI-1 concentrations (discussed later), there were no significant ($P < 0·05$) effects following the 14 d consumption of either test starch.

Glucose and insulin concentrations

Fasting glucose and insulin concentrations, averaged across the four study days, were 4·97 (pooled SEM 0·09) mmol/l and 5·1 (pooled SEM 0·11) mu/l, respectively. There were marked differences in the postprandial plasma glucose and serum insulin profiles, between test starches (Figs 3 and 4; Table 3). Following consumption of the rapidly digestible starch A, concentrations of both glucose and insulin rose more rapidly and to higher peaks than after consumption of

Table 1. Anthropometric characteristics and baseline fasting blood measurement for the ten female volunteers

Measurement	Mean	SE
Age	28·0	1·70
Waist:hip ratio	0·78	0·104
BMI	22·9	0·70
Activity level (h/week)*	13·5	6·52
Fasting serum cholesterol (mmol/l)	4·57	0·145
Fasting plasma triacylglycerols (mmol/l)	0·73	0·085
Fasting plasma glucose (mmol/l)	4·97	0·071

*Activity levels were calculated after each volunteer was asked how many hours a week were spent doing mild, moderate and vigorous activities.

the slowly digestible starch B. The AUC insulin to glucose ratios demonstrated that during the first 120 min of each acute study, starch A evoked a significantly higher insulin response, when compared with the insulin response required for the same quantity of plasma glucose with starch B.

^{13}C *metabolism.* Breath $^{13}CO_2$ output (Fig. 5) was determined as a measure of glucose absorption and oxidation following consumption of the test starches which were naturally enriched with ^{13}C. $^{13}CO_2$ output was consistently higher following consumption of starch A during the first 240 min, after which output began to decline, in contrast with starch B where $^{13}CO_2$ output rose to a plateau at 270 min and remained stable until the end of the study at 360 min. The peak $^{13}CO_2$ output values achieved for each starch were also significantly different ($P = 0.004$) (Table 4), with starch A reaching a peak of 0·0139 mg $^{13}C/min$ (averaged across both study days), compared with starch B which reached a peak of just 0·0105 mg $^{13}C/min$ (average across both study days). Analysis of ^{13}C recovery showed that significantly more labelled carbon was recovered as $^{13}CO_2$ following consumption of starch A, when compared with starch B, after both 120 and 360 min of the study ($P = 0.003$ and $P = 0.016$, respectively).

Plasma NEFA and cholesterol

After the initial 15 min, plasma NEFA concentrations (Fig. 6) fell rapidly after consumption of both test starches. Although lower troughs were reached after consumption of starch A, compared with starch B, the net changes from baseline were not statistically

significant. NEFA concentrations remained well below fasting values until 180 min after the test meals, for both starches, before rising slowly to baseline concentrations for starch B, and rising rapidly to well above fasting concentrations for starch A. These patterns of change were also reflected in the highly significant ($P < 0.001$) differences in time spent below fasting concentrations and AOC between T0 and T120 (Table 5).

Fasting (T0) and end of study (T360) serum cholesterol data are shown in Table 6. Neither test starch had a significant effect on total cholesterol concentrations at either time-point. In addition, HDL:LDL ratio was also unaffected by the treatments.

Haemostasis factors

Plasma concentrations of fibrinogen and PAI-1 were analysed before (T0) and at the end (T360) of each acute study (Table 6). Fibrinogen concentrations were not affected by the consumption of either test starch and remained within a physiologically normal range throughout each acute study. For PAI-1, whilst there was no significant difference in circulating concentrations following consumption of either test starches, the 14 d period of exposure to the test starches reduced significantly postprandial PAI-1 concentrations (Table 6). In the initial acute studies, the T360 PAI-1 values were more than double baseline values for both starches A and B. However, after 14 d consumption of either test starch, whilst there was no difference in the baseline fasting (T0) values, the increase in PAI-1 concentrations at T360 were significantly blunted (by approximately 70 %) for each treatment.

Discussion

The results of epidemiological and experimental studies suggest that the rate and extent of dietary starch digestion can affect a range of physiological indices and modulate the risk of several common non-communicable diseases, in particular those related to insulin resistance (FAO/WHO, 1998; Mathers & Daly, 1998, 2001). In the present study two starches with contrasting digestion patterns were used to assess (1) whether the Englyst *et al.* (2000) method of classifying rapidly and slowly digestible starches reflects glycaemic responses *in vivo*; (2) differences in glycaemic, insulinaemic, lipaemic and haemostasic responses to a test meal; and (3) whether the metabolic consequences of starch

Table 2. Energy and nutrient intakes at baseline and during each of the starch supplementation periods*

	Baseline diet (n 10)		Starch A − 14 d diet (n 9)		Starch B − 14 d diet (n 10)		P for inter-subject variation	P inter-record variation
	Mean	SE	Mean	SE	Mean	SE		
Total energy (kJ/d)	9018[a]	1568	10 346[a]	2173	9427[a]	1923	0·031	0·14
Fat as % of total energy	30·7[a]	6·8	29·0[a]	4·3	28·7	6·3	<0·001	0·36
Protein as % of total energy	13·5[a]	3·4	11·3[a]	4·2	12·5[a]	4·4	0·23	0·45
Total carbohydrate as % of total energy	47·1[a]	9·5	52·5[a]	8·6	50·4[a]	9·8	0·013	0·20
Starch as % of total energy	22·4[a]	4·9	29·4[b]	6·6	28·6[b]	4·2	0·19	0·008
Starch as % of total carbohydrate	48·5[a]	11·0	56·0[a]	8·6	58·6[b]	11·6	0·007	0·018
Recovery of p-amino-benzoic acid (%)	92·6[a]	8·2	102·7[a]	21·7	101·6[a]	17·9	0·96	0·40
Urinary N/N intake	0·93[a]	0·13	0·73[a]	0·19	0·85[a]	0·23	0·75	0·12

[a,b] Mean values within a row with unlike superscript letters were significantly different ($P < 0.05$).
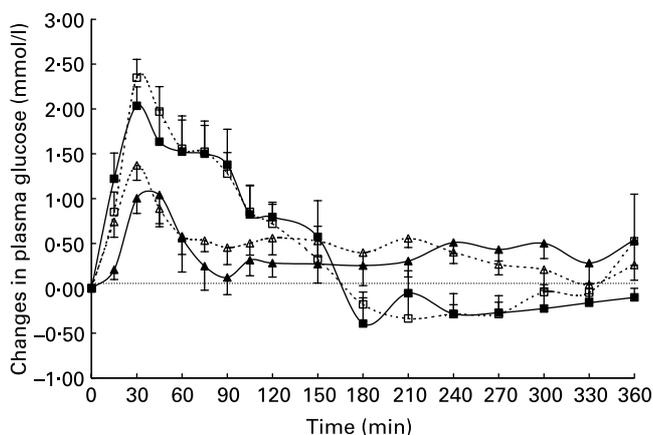*For details of procedures, see p. 949.

Fig. 3. Change from baseline in plasma glucose concentrations after consumption of the rapidly digestible starch A (■ and □, *n* 9) and slowly digestible starch B (▲ and △, *n* 10) in the acute study (—) and following the 14 d period of exposure to the test starches (- - -). Values are means with their standard errors depicted by vertical bars. For details of procedures, see p. 949.

consumption are modulated after a 14 d period of exposure to the test starches.

To date, probably the most widely utilized method of predicting glycaemic responses to starchy foods is the GI, and a list of GI for a range of different foods was compiled by Foster-Powell & Brand-Miller (1995). However, this methodology requires the use of human volunteers and concerns have been raised over the applicability of the GI approach to complex meals (Flint *et al.* 2004) and different population groups. The usefulness of *in vitro* approaches to predict *in vivo* glycaemia remains unclear. Holm & Bjorck (1992) have described procedures for characterizing starch digestion *in vtiro* and made comparisons with *in vivo* metabolic responses whilst Englyst *et al.* (1992, 2000) provide an *in vitro* method for quantifying the rapidly digestible, slowly digestible and resistant starch fractions of starchy foods. Englyst *et al.* (1999) also suggested that rapidly available glucose, determined from the hydrolysis protocol, provides a good indicator of glycaemic response *in vivo*.
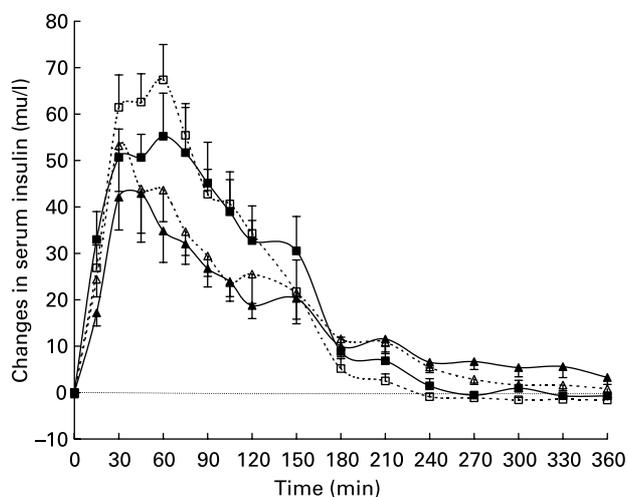

Fig. 4. Change from baseline in serum insulin concentration after consumption of rapidly digestible starch A (■ and □, *n* 9) and slowly digestible starch B (▲ and △, *n* 10) in the acute study (—) and following the 14 d period of exposure to the test starches (- - -). Values are means with their standard errors depicted by vertical bars. For details of procedures, see p. 949.

The results from the present study demonstrate that the *in vitro* data are able to predict the glycaemic response to each test starch in the healthy female volunteers investigated. This is illustrated by a comparison of 120 min AUC ratios for starch A–starch B for glucose released following *in vitro* hydrolysis (1·17:1), with that for the plasma glucose response over the same time period (1·14:1).

As reported in a previous study from our group (Seal *et al.* 2003), starches with different rates of digestion *in vitro* evoke significantly different glycaemic and insulinaemic responses. This is further confirmed in the present study with the rapidly digestible starch A, resulting in more pronounced rises and falls in plasma glucose and in serum insulin concentrations, compared with the slower, more sustained responses to the slowly digestible starch B. The differences between the two test starches during the first 120 min were, however, more exaggerated for the plasma glucose response, when compared with the insulin response. This enhanced insulin response was not reported in the Seal *et al.* (2003) study, however the test meals in that study contained no fat. It is possible that the insulin results reported here may have been affected by the co-ingestion of fat, which has been shown to stimulate early insulin secretion (Collier *et al.* 1984, 1988).

$^{13}CO_2$ output in breath was used as an index of postprandial starch hydrolysis and subsequent glucose absorption and oxidation, although absorbed glucose which is not stored and not oxidized within the time-frame of the study is not measured by this approach. This analysis was possible as both test starches derive from naturally enriched maize (corn), which accumulates excess ^{13}C during CO_2 fixation in the C4 photosynthetic pathway (Taiz & Zieger, 1991). In agreement with the postprandial glycaemia data, the rate of $^{13}CO_2$ output and ^{13}C recovery for starch A were significantly higher than for starch B. The present data are in accord with the observations of Normand *et al.* (1992) and Seal *et al.* (2003), in showing that glucose absorption and oxidation continued long after blood glucose concentrations had fallen back to fasting concentrations (for starch A) and approaching fasting concentrations for starch B. It has been suggested that the observed pattern of $^{13}CO_2$ output is closely related to the pattern of glucose uptake from the gut, after the initial delay resulting from the postprandial surge of insulin, and the switch in overall metabolic oxidation from fat to glucose (Seal *et al.* 2003). The data from the present study, along with others (Achour *et al.* 1997; Daly *et al.* 2000; Vonk *et al.* 2000; Seal *et al.* 2003), demonstrate the utility of this ^{13}C-based approach in investigating the metabolic effects of starches with differing digestion patterns.

In accordance with findings from previous reports (Frayn, 1998; Normand *et al.* 2001; Seal *et al.* 2003), the postprandial changes in NEFA concentrations from the present study correlated inversely with the insulin responses to each test starch. The resulting slower, more sustained level of circulating NEFA in response to the slowly digestible starch B, without the rapid rise in NEFA seen towards the end of the postprandial period following consumption of the rapidly digested starch A, could prove beneficial in lowering circulating NEFA concentrations.

It is documented that insulin resistance and the resulting elevation in NEFA can alter cholesterol concentrations by increasing production of triacylglycerol-enriched VLDL particles and reducing lipoprotein lipase activity (Abate, 2000). Based on previous studies (Itoh *et al.* 1999; Jarvi *et al.* 1999), it was hypothesized that the slowly digestible starch B would reduce cholesterol concentrations when compared with the rapidly digestible starch A. However, the results from the present study show

Table 3. Derived parameters of plasma glucose and insulin responses for subjects following consumption of the test starches*

| | Starch A | | Starch B | | | Significance of effects (P) | | |
	Study 1	Study 2	Study 1	Study 2	Pooled SEM	Starch A v. B	Study 1 v. 2	(Starch A v. B) × (Study 1 v. 2)
Glucose peak value − T0 (mmol/l)	2·43	2·50	1·40	1·52	0·175	<0·001	0·58	0·89
Insulin peak value − T0 (μ/l)	60·6	68·5	57·0	56·9	4·60	0·11	0·40	0·38
AUC insulin (μ/l) to glucose (mmol/l) ratio from T0 to T120	7·08	7·99	6·13	6·90	0·439	0·029	0·06	0·88

AUC, area under the curve.

*Study 1 is the initial acute study; study 2 is the second acute study following the 14 d test starch supplementation period. For details of procedures, see p. 000.
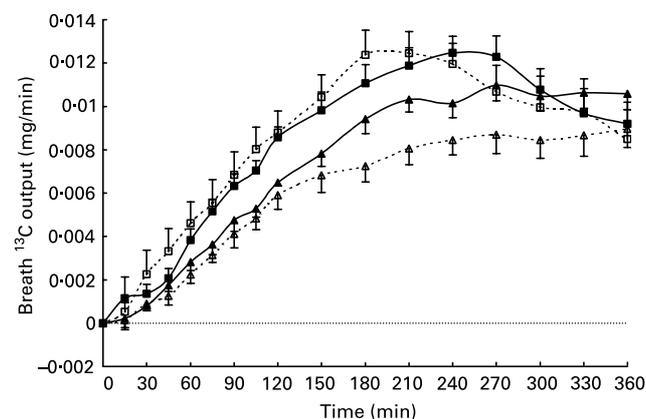

Fig. 5. Change from baseline in breath ^{13}C output after consumption of rapidly digestible starch A (■ and □, n 9) and slowly digestible starch B (▲ and △, n 10) in the acute study (—) and following the 14 d period of adaptation (- - -). Values are means with their standard errors depicted by vertical bars. For details of procedures, see p. 949.
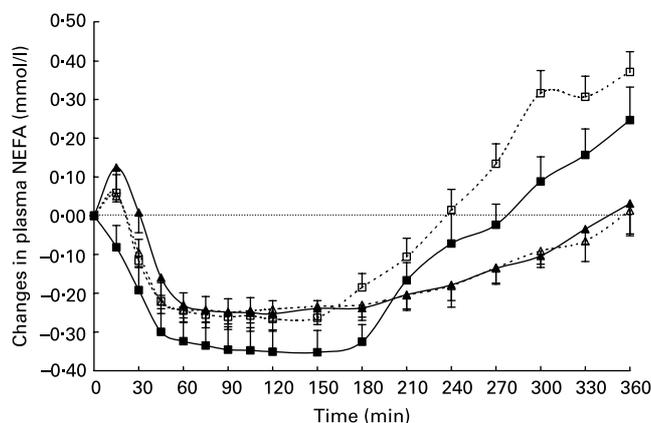

Fig. 6. Change from baseline in plasma NEFA concentrations after consumption of rapidly digestible starch A (■ and □, n 9) and slowly digestible starch B (▲ and △, n 10) in the acute study (—) and following the 14 d period of adaptation (- - -). Values are means with their standard errors depicted by vertical bars. For details of procedures, see p. 949.

that there were no differences between two test starches in either total cholesterol concentrations or HDL:LDL ratio, both during acute starch administration and following 14 d adaptation to the test starches. The present finding may suggest that the young, relatively lean female participants had good insulin sensitivity.

Raised concentrations of fibrinogen and PAI-1 have been linked mechanistically with insulinaemia and CHD development (Bastard *et al.* 2000; Raynaud *et al.* 2000; Kohler, 2002). Although Rauramaa *et al.* (1994) demonstrated a strong inverse correlation between starch intake and fibrinogen levels in older men, neither test starch altered fibrinogen concentrations in the healthy female volunteers that participated in the present study. This result is perhaps unsurprising, since Raynaud *et al.* (2000) reported a highly significant correlation between fibrinogen and insulin sensitivity, and the volunteers in the present study may

have been relatively insulin-sensitive. Further investigation in insulin-resistant and/or diabetic individuals is warranted.

When PAI-1 concentrations are raised, fibrinolytic activity is depressed and there is a subsequent increase in the risk of thrombosis. In the present study, PAI-1 concentrations in the initial acute study for both test starches more than doubled over the postprandial period, when compared with baseline concentrations. Carroll & Schade (2003) also reported a postprandial rise in PAI-1, which tripled from baseline concentrations to 4 h after a high-fat (70 g) meal served to type 2 diabetic participants. The higher fat content of the Carroll study meal may explain the more pronounced rise in postprandial PAI-1 concentrations. However, this theory is contradicted by Sanders *et al.* (2004), who reported a postprandial decrease in PAI-1 concentrations, irrespective of the fat content of the test meal consumed by the non-diabetic, male participants. One possible explanation of

Table 4. ^{13}C recovery in breath carbon dioxide of subjects following consumption of the test starch meal*

| | Starch A | | Starch B | | | Significance of effects (P) | | |
	Study 1	Study 2	Study 1	Study 2	Pooled SEM	Starch A v. B	Study 1 v. 2	(Starch A v. B) × (Study 1 v. 2)
Peak ^{13}C value (μg above background)	0·0138	0·0140	0·0124	0·0087	0·00 030	0·004	0·14	0·11
^{13}C recovery after 120 min (%)	10·0	11·5	7·4	6·9	0·36	0·003	0·65	0·34
^{13}C recovery after 360 min (%)	54·1	56·2	48·2	42·6	4·83	0·016	0·63	0·31

*Study 1 is the initial acute study; study 2 is the second acute study following the 14 d test starch supplementation period. For details of procedures, see p. 949.

Table 5. Derived parameters of plasma NEFA responses following ingestion of the starch test meal*

	Starch A		Starch B			Significance of effects (*P*)		
Response (mmol/l × min)	Study 1	Study 2	Study 1	Study 2	Pooled SEM	Starch A *v.* B	Study 1 *v.* 2	(Starch A *v.* B) × (Study 1 *v.* 2)
Time below fasting	236	198	290	284	15·6	<0·001	0·17	0·31
AOC (T0 to T120)	64	69	36	34	5·2	<0·001	0·73	0·49

AOC, area over the curve.
*Study 1 is the initial acute study; study 2 is the second acute study following the 14 d test starch supplementation period. For details of procedures, see p. 949.

Table 6. Fasting (T0) and 6 h (T360) postprandial values for total cholesterol, HDL:LDL ratio, fibrinogen and plasminogen activator inhibitor-1 (PAI-1)*

	Starch A		Starch B			Significance of effects (*P*)		
Response	Study 1	Study 2	Study 1	Study 2	Pooled SEM	Starch A *v.* B	Study 1 *v.* 2	(Starch A *v.* B) × (Study 1 *v.* 2)
Total cholesterol − T0 (mmol/l)	4·48	4·60	4·69	4·66	0·125	0·28	0·72	0·55
Total cholesterol − T360 (mmol/l)	4·35	4·37	4·46	4·37	0·127	0·64	0·80	0·67
HDL:LDL ratio −T0 (mmol/l)	0·73	0·69	0·72	0·80	0·009	0·58	0·81	0·54
HDL:LDL ratio − T360 (mmol/l)	0·20	0·51	0·18	0·29	0·067	0·51	0·24	0·59
T0 fibrinogen (g/l)	2·5	2·3	2·5	2·4	0·10	0·49	0·18	0·57
T360 fibrinogen (g/l)	2·2	2·2	2·2	2·1	0·06	0·62	0·52	0·90
T0 PAI-1 (ng/ml)	32·5	41·1	29·9	31·8	4·20	0·17	0·21	0·42
T360 PAI-1 (ng/ml)	77·2	55·6	61·7	41·3	9·16	0·11	0·022	0·95

*Study 1 is the initial acute study; study 2 is the second acute study following the 14 d test starch supplementation period. For details of procedures, see p. 949.

these contrasting findings is physical activity levels, which are known to correlate inversely with PAI-1 concentrations (Rankinen *et al.* 1995; Ivey *et al.* 2003). The participants of both the present and the Carroll study were rested in hospital throughout the postprandial sampling period, whilst the participants in the study by Sanders *et al.* (2004) were permitted to return to home or work between sampling. It is therefore likely that the activity levels were higher in the Sanders study, which may have reduced the postprandial PAI-1 concentrations.

A novel observation, previously unreported, is the significant blunting of the observed postprandial rise in PAI-1 concentrations after 14 d of regular test starch consumption, irrespective of the test starch consumed. The design of the present study makes it impossible to determine whether it was increased starch consumption and/or the compensatory decrease in other dietary carbohydrates *per se* which reduced postprandial PAI-1 concentrations, and the observation warrants further investigation.

During the present study, each metabolic parameter was examined before and after a 14 d period of daily test starch consumption, to establish whether habituation to the test starch influenced the metabolic outcomes. However, PAI-1 concentration was the only variable influenced by the regular test starch consumption. Since intestinal and pancreatic enzyme activities are known to respond to changes in dietary composition, it was hypothesized that metabolic 'adaptation' may result in a significant difference in the subsequent glycaemic and insulinaemic responses to each of the test starches. Previous studies have indeed suggested that increased starch intake over time stimulates specific amylase activity (Corring, 1980; Flores *et al.* 1988) and increases the rate of intestinal glucose transport (Ferraris *et al.* 2001). The results from the present study, however, suggest that after the daily consumption of 75 g of either test starch over 14 d, neither fasting nor postprandial glycaemia, insulinaemia, NEFA, cholesterol or fibrinogen were altered significantly.

In conclusion, the results from the present study show that starches with contrasting hydrolysis characteristics *in vitro* have correspondingly different postprandial glycaemic and insulinaemic responses in healthy young female volunteers. The present findings suggest that consumption of slowly digestible starches could improve glycaemic control and lower postprandial NEFA concentrations, which could contribute to prevention and treatment of diabetes and complications of the metabolic syndrome. An increase in starch consumption *per se* may also reduce postprandial PAI-1 concentrations, thus reducing a potential risk factor implicated in the metabolic syndrome.

References

Abate N (2000) Obesity and cardiovascular disease. Pathogenetic role of the metabolic syndrome and therapeutic implications. *J Diabet Comp* **14**, 154–174.

Achour L, Flouie B, Briet F, *et al.* (1997) Metabolic effects of digestible and partially indigestible cornstarch: a study in the absorptive and postabsorptive periods in healthy humans. *Am J Clin Nutr* **66**, 1151–1159.

Argiles JM & Lopez-Soriano FJ (2001) Insulin and cancer (review). *Int J Oncol* **18**, 683–687.

Bastard JP, Pieroni L & Hainque B (2000) Relationship between plasma Plasminogen activator inhibitor 1 and insulin resistance. *Diabetes Metab Res Rev* **16**, 192–201.

Bingham SA & Cummings JH (1983) The use of 4-aminobenzoic acid as a marker to validate the completeness of 24h urine collection in man. *Clin Sci* **64**, 629–635.

Bingham SA & Cummings JH (1985) Urine nitrogen as an independent validatory measure of dietary intake: a study of nitrogen balance in individuals consuming their normal diet. *Am J Clin Nutr* **42**, 1276–1289.

Bornet FRJ, Fontvielle A-M, Rizkalla S, *et al.* (1989) Insulin and glycaemic responses in healthy humans to native starches processed in different ways; correlation with in vitro α-amylase hydrolysis. *Am J Clin Nutr* **50**, 315–323.

Carroll MF & Schade DS (2003) Timing of antioxidant vitamin ingestion alters postprandial proatherogenic serum markers. *Circulation* **108**, 24–31.

Champ M, Martin L, Noah L & Gratus M (1999) Analytical methods for resistant starch. In *Complex Carbohydrates in Foods*, pp. 169–187 [SS Chol, L Prosky and M Dreher, editors]. New York: Marcel Dekker.

Collier GR, Greenberg GR, Wolever TMS & Jenkins DJA (1988) The acute effect of fat on insulin secretion. *J Clin Endocrinol Metab* **66**, 323–326.

Collier G, McLean A & O'Dea K (1984) Effect of co-ingestion of fat on the metabolic responses to slowly and rapidly absorbed carbohydrates. *Diabetologia* **26**, 50–54.

Corring T (1980) The adaptation of digestive enzymes to the diet: its physiological significance. *Reprod Nutr Dev* **20**, 4B, 1217–1235.

Daly ME, Vale C, Littlefield A, Alberti KGMM & Mathers JC (2000) Acute fuel selection in response to high-sucrose and high-starch meals in healthy men. *Am J Clin Nutr* **71**, 1516–1524.

Daly ME, Vale C, Walker M, Littlefield A, Alberti KGMM & Mathers JC (1998) Acute effects on insulin sensitivity and diurnal metabolic profiles of a high-sucrose compared with a high-starch diet. *Am J Clin Nutr* **67**, 1186–1196.

Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects* no. 55. London: HMSO.

Englyst HN, Kingman SM & Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. *Eur J Clin Nutr* **46**, Suppl. 2, S33–S50.

Englyst HN, Kingman SM, Geoffrey JH & Cummings JH (1996) Measurement of resistant starch in vitro and in vivo. *Br J Nutr* **75**, 749–755.

Englyst HN, Veenstra J & Hudson GJ (1996) Measurement of rapidly available glucose (RAG) in plant foods: a potential in vitro predictor of the glycaemic response. *Br J Nutr* **73**, 327–337.

Englyst KN, Englyst HN, Hudson GJ, Cole TJ & Cummings JH (1999) Rapidly available glucose in foods: an in vitro measurement that reflects the glycaemic response. *Am J Clin Nutr* **69**, 448–454.

Englyst KN, Hudson GJ & Englyst HN (2000) Starch analysis in food. In *Encyclopaedia of Analytical Chemistry*, pp. 4246–4262 [RA Meyers, editor]. Chichester: John Wiley & Sons.

Facchini FS, Hua NW, Reaven GM & Stoohs RA (2000) Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? *Free Radic Biol Med* **29**, 1302–1306.

FAO/WHO (1998) *Carbohydrates in Human Nutrition. Report of a Joint FAO/WHO Report, Rome, 14–18 April 1997*, paper 66. Rome: FAO.

Ferraris RP (2001) Dietary and developmental regulation of intestinal sugar transport. *Biochem J* **360**, part 2, 265–276.

Flint A, Koller BK, Raben A, *et al.* (2004) The use of glycaemic index tables to predict glycaemic index of composite breakfast meals. *Br J Nutr* **91**, 979–989.

Flores CA, Brannon PM, Bustamante SA, *et al.* (1988) Effect of diet on intestinal and pancreatic enzyme activities in the pig. *J Pediatr Gastroenterol Nutr* **7**, 914–921.

Foster-Powell K & Brand-Miller J (1995) International tables of glycaemic index. *Am J Clin Nutr* **62**, 871S–893S.

Frayn KN (1998) Non-esterified fatty acid metabolism and postprandial lipaemia. *Atherosclerosis* **141**, Suppl. 1, S41–S46.

Holm J & Bjorck I (1992) Bioavailability of starch in various wheat-based bread products: evaluation of metabolic responses in healthy subjects and rate and extent of in vitro starch digestion. *Am J Clin Nutr* **55**, 420–429.

Itoh K, Imai K, Masuda T, *et al.* (1999) Relationship between serum total cholesterol level and nutritional status in Japanese young female. *Nutr Res* **19**, 1145–1152.

Ivey FM, Womack CJ, Kulaputana O, Dobrovovolny CL, Wiley LA & Macko RF (2003) A single bout of walking exercise enhances endogenous fibrinolysis in stroke patients. *Med Sci Sports Exerc* **35**, 193–198.

Jarvi AE, Karlstrom BE, Granfeldt YE, Bjorck IE, Asp N-G & Vessby BOH (1999) Improved glycaemic control and lipid profile and normalized fibrinolytic activity on a low-glycaemic index diet in type 2 diabetic patients. *Diabetes Care* **22**, 10–18.

Jenkins DJA, Kendall CW, Augustin LS, *et al.* (2002) Glycaemic index: overview of implication in health and disease. *Am J Clin Nutr* **76**, 226S–273S.

Jenkins DJA, Wolever TMS, Taylor RH, *et al.* (1981) Glycaemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr* **34**, 362–366.

Kohler HP (2002) Insulin resistance syndrome: interaction with coagulation and fibrinolysis. *Swiss Med Wkly* **132**, 241–252.

Mathers JC & Daly ME (1998) Dietary carbohydrate and insulin sensitivity. *Curr Opin Clin Nutr Metab Care* **1**, 553–557.

Mathers JC & Daly ME (2001) Food polysaccharides, glucose absorption and insulin sensitivity. In *Advanced Dietary Fibre Technology*, pp. 186–196 [DBV McCleary and L Prosky, editors]. Oxford: Blackwell Science.

Nelson M, Atkinson M & Meyer J (1997) *A Photographic Atlas of Food Portion Sizes*. London: MAFF Publications.

Normand S, Khalfallah Y, Louche-Pelissier C, *et al.* (2001) Influence of dietary fat on postprandial glucose metabolism (exogenous and endogenous) using intrinsically ^{13}C-enriched durum wheat. *Br J Nutr* **86**, 3–11.

Normand S, Rachiaudi C, Khalfallah Y, Guilluy R, Mornex R & Riou JP (1992) ^{13}C appearance in plasma glucose and breath CO_2 during feeding with naturally ^{13}C-enriched starch food in normal humans. *Am J Clin Nutr* **55**, 430–435.

Rankinen T, Vaisanen S, Penttila I & Rauramaa R (1995) Acute dynamic exercise increases fibrinolytic activity. *Thromb Haemost* **73**, 281–286.

Rauramaa R, Vaisanen S, Penttila I & Rankinen T (1994) Inverse relationship between dietary starch intake and plasma fibrinogen in middle-aged men. *Nutr Metab Cardiovasc Dis* **4**, 192–196.

Raynaud E, Perez-Martin A, Brun JF, Aissa-Benhaddad A, Fedou C & Mercier J (2000) Relationships between fibrinogen and insulin resistance. *Atherosclerosis* **150**, 365–370.

Reaven GM (1995) Pathophysiology of insulin resistance in human disease. *Physiol Rev* **75**, 473–486.

Sanders TAB, de Grassi T, Acharya J, Miller GJ & Humphries SE (2004) Postprandial variations in fibrinolytic activity in middle-aged men are modulated by Plasminogen activator inhibitor I 4G-675/5G genotype but not by the fat content of a meal. *Am J Clin Nutr* **79**, 577–581.

Seal CJ, Daly ME, Thomas LC, Bal W, Birkett AM, Jeffcoat R & Mathers JC (2003) Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined in vitro. *Br J Nutr* **90**, 853–864.

Taiz L & Zeiger E (1991) *Plant Physiology*, pp. 234–224. Redwood City, CA: Benjamin/Cummings Publishing Co.

Vonk RJ, Hagedoorn RE, De Graaff R, *et al.* (2000) Digestion of so-called resistant starch sources in the human small intestine. *Am J Clin Nutr* **72**, 432–438.

Wallace TM & Matthews DR (2002) The assessment of insulin resistance in man. *Diabet Med* **19**, 527–534.

Wolever TMS (2000) Dietary carbohydrates and insulin action in humans. *Br J Nutr* **83**, S97–S102.

Yoshikawa T, Yoshikazu N, Doi C, Makino T & Nomura K (2001) Insulin resistance in patients with cancer: relationships with tumor site, tumor stage, body-weight loss, acute-phase response, and energy expenditure. *Nutrition* **17**, 590–593.