

## Postprandial carbohydrate metabolism in healthy subjects and those with type 2 diabetes fed starches with slow and rapid hydrolysis rates determined *in vitro*

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The objective of the present study was to investigate the effects of starches with differing rates of hydrolysis on exposure to pancreatin *in vitro* on postprandial carbohydrate metabolism in healthy subjects and in subjects with type 2 diabetes. Two test starches, prepared from uncooked native granular starch products, and naturally enriched with <sup>13</sup>C, were consumed in a randomized crossover design by eight healthy and thirteen type 2 diabetic subjects. One starch was characterized *in vitro* as being rapidly hydrolysed (R, 94% after 180 min), and the other was more slowly hydrolysed (S, 51% after 180 min). Each subject consumed 50 g of each test starch. In addition, the type 2 diabetic subjects consumed 89.7 g of the S starch on a separate occasion. Blood samples were taken at 10 min intervals for 3 h, and at 20 min intervals for a further 3 h during a 6 h postprandial period. Breath <sup>13</sup>CO<sub>2</sub> enrichment was measured at the same time points, and indirect calorimetry was performed for seven 20 min sessions immediately before and during the 6 h postprandial period. With the R starch, plasma glucose concentrations and serum insulin concentrations rose faster and the maximum glucose change was approximately 1.8 times that for the S starch, averaged across both subject groups. The areas under the curves for glucose and insulin were, respectively, 1.7 and 1.8 times higher for the R starch compared with the S starch, averaged across both subject groups. The rate of <sup>13</sup>CO<sub>2</sub> output and the proportion of <sup>13</sup>C recovered in breath after consumption of the R starch was similar for both subject groups. The results provide evidence that starches which have different rates of hydrolysis *in vitro* result in different patterns of glycaemia and insulinaemia in both healthy adults and in diet-controlled type 2 diabetic subjects. Data from the hydrolysis of novel starch products *in vitro*, therefore, are useful in predicting glycaemic responses *in vivo*.

### Starch digestion: Glycaemia: Insulinaemia: *In vitro* starch hydrolysis: Diabetes

For the maintenance of health and prevention of several common non-communicable diseases in adults, the recent FAO/WHO Expert Consultation (Food and Agriculture Organization/World Health Organization, 1998) recommended: 'that energy balance be maintained by consuming a diet containing at least 55% of total energy from carbohydrate from various sources...'. The recommendation underlines the importance of starchy foods derived from cereals, tubers and roots in healthy diets and emphasizes the diversity of metabolic and health responses to different food carbohydrates. A key component of these metabolic responses is the rate and extent of glucose absorption with corresponding requirements for insulin secretion. Sub-optimal glucose tolerance and insulin insensitivity appear to play important roles in the aetiology of diabetes

and cardiovascular disease (Reaven, 1995) and may be modulated by dietary carbohydrate consumption patterns (Daly *et al.* 1997; Mathers & Daly, 1998). The amount of dietary carbohydrate in the diet and the rate and extent of digestion of this carbohydrate are the principal determinants of postprandial glucose and insulin responses (Wolever & Bolognesi, 1996; Wolever, 2000). Predicting these responses, however, has proved difficult. Prediction relies upon a combination of *in vitro* procedures to classify carbohydrates according to their rate of hydrolysis and release of glucose (Englyst *et al.* 1992, 1996, 1999; Åkerberg *et al.* 1998), and *in vivo* measurement of glycaemic index (GI) (Jenkins *et al.* 1981, 2002; Wolever & Jenkins, 1986; Bornet *et al.* 1989; Wolever, 2000). In addition, studies using carbohydrates labelled with stable isotopes have

**Abbreviations:** GI, glycaemic index; IAUC, incremental area under the curve; NEFA, non-esterified fatty acids; R, rapidly hydrolysed; S, slowly hydrolysed.

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been used to make direct comparisons between *in vitro* rates of hydrolysis and glycaemic response in human subjects (Normand *et al.* 1992; Vonk *et al.* 2000) and pigs (Noah *et al.* 2000).

Recent advances in the understanding of the roles of starches in health have identified the need to develop food starches which differ in their rate and extent of digestion in the small intestine (Wolever, 1991; Jenkins *et al.* 2002). Consumption of these starches may help in the management of diabetes through reduction in the excursions in plasma glucose and insulin concentrations. Such slowly digested starchy foods may be of wider benefit in reducing the risk of some common non-transmissible diseases in the general public. Incorporation of appropriately characterized starches into foods may provide a palatable and acceptable way of eating slowly digestible starches. The aim of the present series of studies was to investigate the effects of two contrasting uncooked native granular starches, naturally enriched with  $^{13}\text{C}$  and with differing rates of hydrolysis *in vitro*, on postprandial glycaemia and insulinaemia in normal healthy adults and in subjects with type 2 (non-insulin-dependent) diabetes.

## Materials and methods

### Subjects

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

Eight healthy, weight-stable volunteers (four male and four female) were recruited from the student and staff populations of the University of Newcastle upon Tyne and from staff of the Royal Victoria Infirmary, Newcastle upon Tyne. None had diabetes mellitus (or a first-degree relative with diabetes), IHD, hypertension, or any other disease associated with altered insulin sensitivity. None were taking any drugs known to alter insulin sensitivity or to affect carbohydrate or lipid metabolism. All were non-smokers.

Thirteen volunteers (nine male and four female) diagnosed with type 2 diabetes controlled by diet alone were recruited using the patient database in the Newcastle upon Tyne Diabetes Centre, Newcastle General Hospital, Newcastle upon Tyne. Exclusion criteria for recruitment included: poor diabetes control (glycated haemoglobin  $>8.5\%$ ); major complications of diabetes; use of sulfonylurea or  $\alpha$ -1-glucosidase inhibitor therapy; a BMI  $>30\text{ kg m}^2$  recorded in the patient's hospital notes.

### The experimental starches

The experimental starches were uncooked native granular starches not derived via genetic engineering or by chemical modification, and were provided by the National Starch and Chemical Company (Bridgewater, NJ, USA). Raw starches were chosen to investigate whether starches with

different rates of digestion *in vitro* result in different physiological responses *in vivo*. Although not common in the diet, raw starches are eaten by human consumers; for example, in muesli, and raw maize starch is currently used in minimally processed products for diabetics, and is recommended for patients with certain forms of glycogen storage disease. To the authors' knowledge, the starches used in the present study have not been investigated in this way before. *In vitro* hydrolysis of the test starches was determined as follows. Starch (2 g) was mixed with 40 ml phosphate buffer (0.05 M, pH 6.9; 0.68 M-NaCl) and equilibrated at 37°C. At time zero, 5% (w/v) pancreatin (8 ml) (Sigma, catalogue no. P-7545; St Louis, MO, USA) in phosphate buffer was added to the starch suspension. Samples (1.5 ml) of the digestion mixture were removed at 10 min intervals for 60 min, 20 min intervals for the next 120 min and at 60 min intervals thereafter to a total of 360 min into microcentrifuge tubes and centrifuged for 2 min at 9000g (Fischer Scientific Microcentrifuge 59V; Pittsburgh, PA, USA). After enzymic digestion the hydrolysis products in the supernatant fraction were measured as percentage solubles using a Fischer Handheld Refractometer (Fischer Scientific; Pittsburgh, PA, USA) with a Brix scale that had been zeroed with distilled water. The Brix scale measures sugar concentrations in solutions. The percentage solubles value was then converted to percentage starch digested by accounting for the percentage solubles contributed by all components before incubation, as well as the initial weight of starch digested. As a further comparison of the test starches, the resistant starch content of each product was determined using the procedure described by Englyst *et al.* (1992), with additional measurements of starch remaining at 360 min. The test starches were the rapidly hydrolysed (R) starch AMIOCA™, and the slowly hydrolysed (S) NUTRA-STAR™. These were presented as pre-weighed samples with a sweetened orange flavouring in screw-topped plastic containers (colour coded) suitable for reconstituting the suspensions immediately before use in each study. The  $^{13}\text{C}$  content of the starches, determined by isotope ratio MS, was higher for the R starch than for the S starch (1.0993 v. 1.0920 atoms percent). The R starch is a single-component waxy maize-derived starch ( $<1\%$  protein,  $<1\%$  fibre, and  $>99\%$  carbohydrate with an amylopectin composition  $>98\%$ ). The S starch is composed of a blend of tapioca and maize starch in the proportion of 46:54 ( $<1\%$  protein,  $<2\%$  fibre and  $>99\%$  carbohydrate with an amylopectin content of at least 80%). The tapioca starch is essentially 'unlabelled' since this component is derived from a non-C4 plant. Since this component of the S starch is 'invisible' using the  $^{13}\text{C}$  enrichment approaches, the quantification of oxidation of exogenous glucose has been restricted to the R starch.

### Experimental protocol: healthy subjects

Each subject ( $n=8$ ) took part in two experimental periods in a randomized, double-blind, crossover design. Subjects were admitted at 07.45 hours, having fasted from 21.00 hours the previous evening. Alcohol and strenuous exercise

were avoided for 24 h before each experimental period. Study periods were separated by a minimum of 1 week (males and post-menopausal females), with 1 month for menstruating females to keep as close as possible to a fixed point in the menstrual cycle. After consumption of 50 g of each test starch suspended in 250 ml cold water, blood samples were taken over a 6 h period from a retrograde cannula in a dorsal hand vein at 10 min intervals for 3 h, and then at 20 min intervals for the next 3 h. The cannula was inserted 15 min before baseline samples were taken, and the hand was kept in a heated box at 55°C to facilitate arterial blood sampling. Breath samples were taken at the same time as blood samples and indirect calorimetry measurements were made using a Deltatrac Indirect Calorimeter (Datex Instrumentarium Corporation, Helsinki, Finland) for 20 min at 40 min intervals from time -20 min to time 100 min and then for 20 min at 60 min intervals until the end of the 6 h study period. Starch suspensions were consumed within 3 min, and volunteers were allowed unlimited access to drinking water throughout the experimental period.

#### *Experimental protocol: type 2 diabetic subjects*

Each subject ( $n$  13) took part in two experimental periods in a randomized, double-blind, crossover design as described for healthy subjects except that in addition they were provided with a standard meal for consumption the evening before each experimental study day in an attempt to reduce variability in fasting blood glucose concentrations. The energy content of the meal was approximately 2.2 kJ, with 38 % energy from fat, 47 % energy from carbohydrate, and 15 % energy from protein. Eleven of the thirteen subjects (eight male, three female; two subjects were withdrawn from the study for unrelated health reasons) took part in a third study of similar design in which the dose of the S starch was increased in an attempt to mimic the glycaemic response to the lower dose of the R starch. The period between the second and third experimental days averaged 10.7 weeks. The quantity of starch consumed in the third experimental period was calculated using the incremental area under the curve (IAUC) as follows:

$$\left( 50 \times \frac{\text{IAUC for the R starch}}{\text{IAUC for the S starch}} \right).$$

The ratio of the areas under the glucose curve for the R and S starches (1.794:1, equivalent to 87.9 g S starch) from eleven of the thirteen diabetic subjects (those who had completed the study at the time of the calculation) was used in the calculation. Volunteers consumed the larger mass of the S starch suspended in 250 ml water, but were asked to drink a further 150–200 ml water after consuming the starch to maintain a similar starch:water value to that used in the earlier studies.

#### *Analytical methods*

Plasma glucose, non-esterified fatty acids (NEFA) and serum insulin concentrations were determined using standard enzymic procedures as described previously (Daly *et al.* 1998, 2000). Enrichment of  $^{13}\text{C}$  in breath

and the enrichment of  $^{13}\text{C}$  in the test starches were determined by isotope ratio MS using an ANCA 20:20 mass spectrometer (Europa Scientific, Crewe, Ches., UK).

#### *Habitual dietary intake of subjects and anthropometric measurements*

The subjects' habitual food intakes were estimated from a 7 d food record collected before the clinical study days and food portion sizes were quantified with the aid of a food atlas (Nelson *et al.* 1997) and face-to-face interview with the subjects on completion of the record. Nutrient intakes were calculated from food records using an Integrated Dietary Analysis program (IDA Publications, London, UK). The food record was validated by comparing estimated food N intake with 24 h urinary N excretion and the completeness of the urine collection was assessed through urinary recovery of a 240 mg oral dose of *p*-amino-benzoic acid (Bingham & Cummings, 1983).

Percentage body fat was calculated by the equations of Siri (1956) from estimates of body density derived from skinfold thickness measurements (using a Holtain-Tanner and Whitehouse skin calliper; Holtain, Crosswell, Pembrokeshire, UK) made over the triceps muscle (Durnin & Womersley 1974).

#### *Calculations and statistical analysis*

The IAUC for plasma glucose response was calculated using the difference between plasma glucose concentration at baseline and each time point using a standard trapezoid procedure (Food and Agriculture Organization/World Health Organization, 1998). Since serum insulin concentrations are not normally distributed, the IAUC for serum insulin was calculated using similar trapezoid procedures using log-transformed data followed by back-transformation for presentation of the results. The incremental area over the curve for the plasma NEFA response was calculated using the difference between the plasma NEFA concentration at baseline and each time point using the same trapezoid procedure. The rate of excretion of  $^{13}\text{C}$  as  $^{13}\text{CO}_2$  in breath was calculated as the product of  $^{13}\text{CO}_2$  enrichment (atoms percent above background) and  $\text{CO}_2$  production (determined by indirect calorimetry at each time point during the experimental period) as described previously (Daly *et al.* 2000). The cumulative output of  $^{13}\text{C}$  over the 360 min experimental period was calculated from the IAUC for  $^{13}\text{CO}_2$  output and expressed as a percentage of the original dose above background for each individual. Carbohydrate and fat oxidation rates, and oxidation of exogenous carbohydrate were calculated from indirect calorimetry data and  $^{13}\text{CO}_2$  enrichment as described by Jeukendrup *et al.* (1997).

Statistical analysis was carried out using SPSS for Windows (SPSS Inc., Chicago, IL, USA). All data within each study were examined using two-tailed paired *t* tests. Differences between data for healthy and type 2 diabetic subjects were compared by ANOVA. Data are presented as mean values and standard deviations.

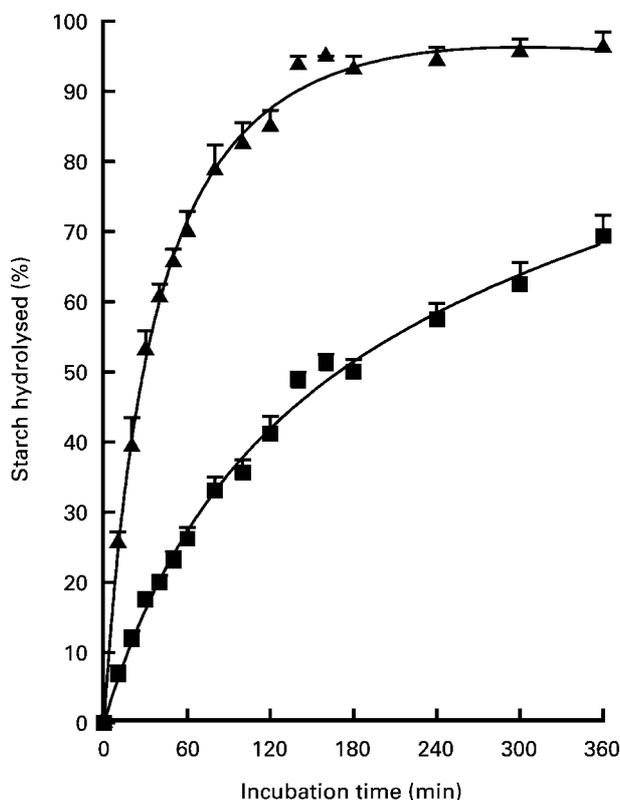
## Results

### *In vitro* starch hydrolysis

The two starches demonstrated contrasting hydrolysis characteristics (Fig. 1). The hydrolysis of the R starch, extracted from waxy maize, was initially very rapid with approximately 94% of the starch hydrolysed after 180 min. The rate of hydrolysis decreased thereafter with approximately 96% hydrolysed after 360 min. In contrast, the S starch, a combination of tapioca and maize starches, was more slowly hydrolysed with only 51% hydrolysis after 180 min, and approximately 68% hydrolysed after 360 min. When determined using the method described by Englyst *et al.* (1992), the resistant starch content of the test starches at 120 min was 11.2 and 31.8%, respectively for the R and S starch. Further measurements of starch remaining at 360 min were 0.0 and 15.0%, respectively for the R and S starch. These observations confirmed the present study's predictions that the R starch would be rapidly hydrolysed, and that the combination of starches in the S starch would be more slowly hydrolysed.

### Subject characterization

The healthy volunteers for the present study were, on average, slightly younger and approximately 10 kg lighter ( $P=0.054$ ) than the type 2 diabetic volunteers but otherwise the two groups were of similar BMI and body composition (Table 1). Fasting plasma cholesterol concentrations were



**Fig. 1.** Percentage of starch hydrolysis assessed *in vitro* ( $n=4$ ) for rapidly hydrolysed ( $\blacktriangle$ ) and slowly hydrolysed ( $\blacksquare$ ) starch. Values are means, with standard errors of the mean represented by vertical bars. For details of procedures, see p. 854.

similar for both groups, but the plasma triacylglycerol concentration of the diabetic group was significantly higher than that of the healthy group (Table 1). The habitual dietary intake data suggested that the mean intakes of carbohydrates and fat (Table 2) were similar to those reported for the adult population of the UK (Gregory *et al.* 1994). In both subject groups, men consumed a higher proportion of energy as alcohol but a lower proportion as carbohydrates than did women. Comparison of reported energy intakes with calculated BMR gave physical activity levels of 1.6 and 1.5 for the healthy subjects and diabetics, respectively, well within accepted ranges for accurate recording of dietary intake (Goldberg *et al.* 1991). Urine collections over 24 h were judged to be complete on the basis of *p*-aminobenzoic acid recovery (Bingham & Cummings, 1983). Both the estimates of physical activity level and reported N intake:urinary N output values indicated that the self-reported food consumption data may be considered as reliable estimates of habitual intake (Bingham & Cummings, 1985; Goldberg *et al.* 1991).

### Plasma metabolite responses with 50 g of the test starches

Fasting plasma glucose concentrations were 5.36 (SD 0.425) and 6.88 (SD 1.737) mM for the healthy subjects and type 2 diabetic subjects, respectively. Fasting plasma insulin concentrations were 6.26 (SD 1.89) and 23.5 (SD 34.94) pM for the healthy subjects and type 2 diabetic subjects, respectively. There were no significant differences between study days for either group for glucose or insulin. There were marked differences in the postprandial plasma glucose and serum insulin profiles between the test starches and between the healthy volunteers and type 2 diabetic subjects (Figs. 2 and 3). With the R starch, plasma glucose rose more rapidly to a higher peak (maximum glucose change) than after consuming the S starch when the glucose response was slower and more sustained (Table 3). For the healthy subjects, plasma glucose concentrations fell slightly below fasting values from approximately 210 min after consuming the R starch and reached fasting values approximately 270 min after consuming the S starch. This is in contrast with the results for the type 2 diabetic subjects in whom the plasma glucose response was, on average, greater and more sustained than that seen for the healthy subjects. In addition, for the diabetic subjects, the plasma glucose concentration reached fasting values after approximately 220–260 min and then continued to fall throughout the measurement period. The area under the glucose curve was higher for the R starch compared with the S starch for both subject groups but significantly so ( $P<0.05$ ) for the diabetics only. The pattern of serum insulin response was similar to that observed for glucose except for a less pronounced fall below baseline concentrations in the late postprandial period with the diabetic subjects.

The plasma NEFA concentrations of the healthy subjects fell rapidly after consuming both starches and reached lower concentrations in subjects consuming the R starch compared with the S starch (0.02 v. 0.08 mM;  $P=0.05$  for the R and S starches, respectively). The response in the type 2 diabetics was similar, but the fall was less rapid and the lowest concentration was higher than that observed

**Table 1.** Anthropometric characteristics and fasting plasma lipid concentrations of the volunteers (Mean values and standard deviations)

	Healthy subjects						Diabetic subjects					
	Males (n 4)		Females (n 4)		Overall (n 8)		Males (n 9)		Females (n 4)		Overall (n 13)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	39.5	15.9	48.5	10.2	44.1	13.4	56.7	8.1	44.3	3.7	52.9	9.1
Body weight (kg)	79.2	5.6	63.3	12.3	71.2	12.3	79.7	10.9	85.7	10.1	81.6	10.6
BMI (kg/m <sup>2</sup> )	25.6	1.5	24.3	3.2	25.9	1.5	26.4	2.3	32.6	3.4	28.3	3.9
Fasting total cholesterol (mmol/l)	4.89	0.47	4.89	0.38	4.89	0.39	4.83	0.57	4.33	1.15	4.67	0.78
Fasting HDL-cholesterol (mmol/l)	1.31	0.20	1.63	0.49	1.47	0.39	1.26	0.31	1.03	0.25	1.18	0.30
Fasting LDL-cholesterol (mmol/l)	3.09	0.30	2.79	0.23	2.94	0.29	2.89	0.69	2.53	0.38	2.78	0.70
Fasting triacylglycerol (mmol/l)	1.10	0.21	1.03	0.43	1.06	0.32	1.53	0.35	1.70	0.93	1.59*	0.55
Hb1Ac (% total haemoglobin)	—	—	—	—	—	—	6.92	0.76	5.85	0.33	6.59	0.82
Time since diagnosis (months)	—	—	—	—	—	—	91	72	105	57	96	65
Waist:Hip	0.89	0.05	0.74	0.06	0.82	0.09	0.92	0.05	0.81	0.03	0.88	0.07
Percentage body fat	29.2	4.8	37.4	3.1	33.3	5.8	28.3	4.7	39.8	2.1	31.8	6.8

Hb1Ac, glycated haemoglobin.

\* Mean value was significantly different from that for the healthy subjects ( $P < 0.025$ ).**Table 2.** Habitual daily energy and nutrient intakes of the volunteers\* (Mean values and standard deviations)

	Healthy subjects						Diabetic subjects					
	Males (n 4)		Females (n 4)		Overall (n 8)		Males (n 9)		Females (n 4)		Overall (n 13)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (MJ)	11.8	2.3	8.9	0.5	10.4	2.2	11.9	2.6	8.7	4.6	10.9	3.5
Fat (% energy)	34.3	6.1	40.5	4.1	37.4	5.9	36.2	6.3	33.9	8.3	35.5	6.7
Total carbohydrate (% energy)	38.5	5.1	44.5	5.1	41.5	5.7	41.8	7.1	48.4	7.8	43.8	7.6
Protein (% energy)	15.0	2.9	12.8	1.3	13.9	2.4	15.7	3.8	17.5	4.7	16.3	3.9
Alcohol (% energy)	12.0	2.8	2.3	2.2	7.1	5.7	6.3	9.1	0.5	0.9	4.5	7.9
Calculated BMR† (MJ/d)	7.06	0.44	5.30	0.32	6.52	1.04	7.23	0.65	6.45	0.34	6.99	0.67
Physical activity level‡	1.59	0.28	1.59	0.17	1.59	0.22	1.56	0.35	1.33	0.32	1.49	0.45
Percentage recovery PABA§	104.8	5.4	98.3	8.4	101.5	7.4	83.4	13.5	86.9	5.7	84.3	11.6
Urinary N/N intake	0.79	0.10	0.80	0.06	0.80	0.08	0.94	0.15	0.92	0.30	0.93	0.19

PABA, *p*-aminobenzoic acid.

\* For details of subjects and procedures, see Table 1 and p. 854.

† Calculated BMR from Schofield equations (Department of Health, 1991).

‡ Reported energy intake/calculated BMR.

§ Oral marker recovered in urine as a measure of completeness of urinary collection (Bingham and Cummings, 1983).

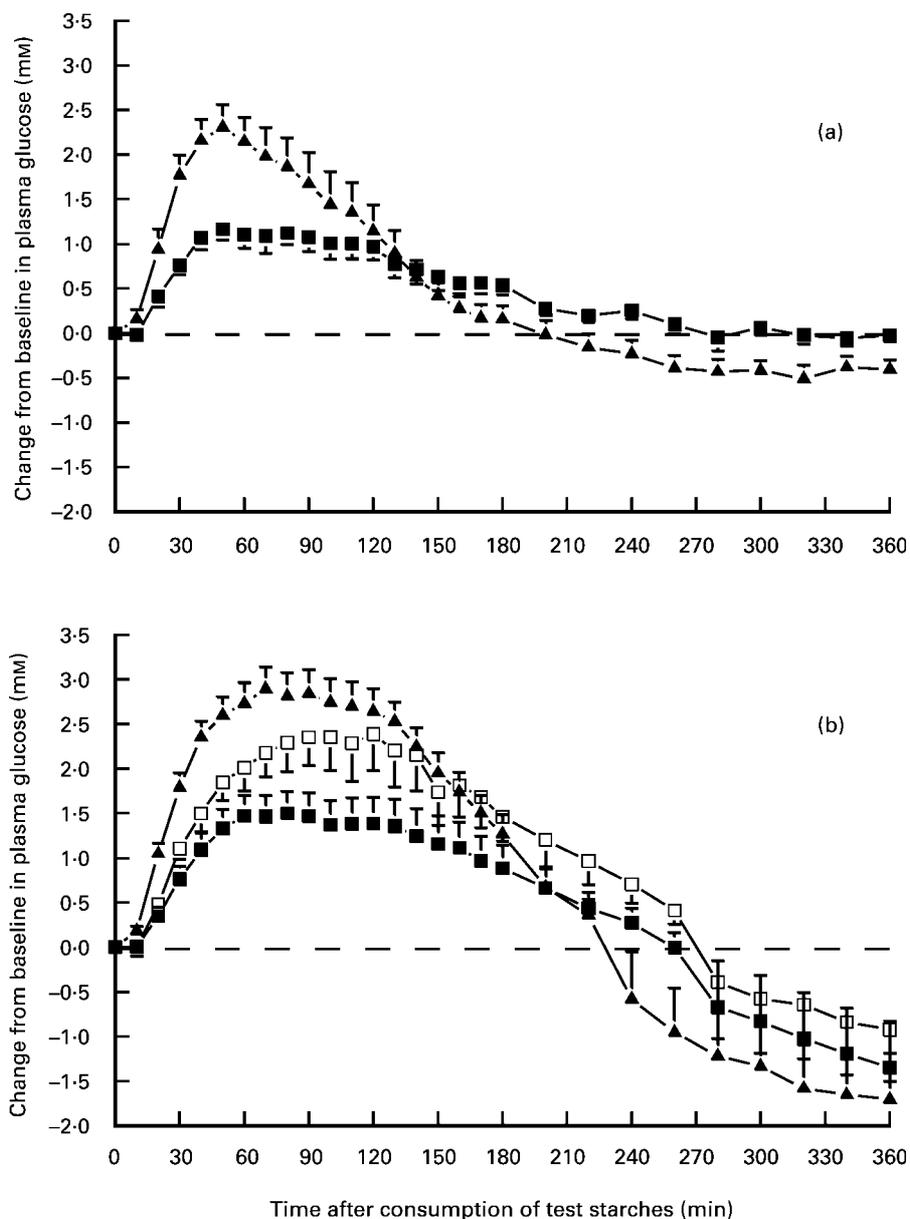
|| Based on average reported N intake on the day before and the day of urine collection.

for the healthy subjects (0.12 v. 0.18 mM for the R and S starches, respectively; Table 3), although this difference was not statistically significant. From approximately 90–150 min after consuming the test starches, plasma NEFA concentrations remained low for 2–3 h before rising above baseline values by the end of the measurement period. During this late postprandial period, the plasma NEFA concentration was higher after consuming the R starch than the S starch in both subject groups. The area over the curve, measured over 360 min, was similar for both starches and for both subject groups (overall mean – 86 mM × min; Table 3). A similar pattern was observed for plasma 3-hydroxybutyrate concentrations, which fell rapidly from fasting concentrations of 0.09 (SD 0.06) and 0.11 (SD 0.094) mM in the healthy subjects and type 2 diabetic subjects, respectively, after consuming the test starches. The plasma concentrations of this metabolite rose more rapidly during the late postprandial period following the consumption of the R starch in both subject

groups. For the healthy subjects, the 3-hydroxybutyrate concentration at 360 min was 0.32 (SD 0.31) mM for the R starch and 0.19 (SD 0.18) mM for the S starch ( $P = 0.356$ ). For the type 2 diabetic subjects, the 3-hydroxybutyrate concentration at 360 min was similar to that seen in the healthy subjects after consuming the R starch. However, the 3-hydroxybutyrate concentration was significantly higher ( $P < 0.05$ ) than for both doses of the S starch (0.32 (SD 0.30) mM compared with 0.10 (SD 0.10) and 0.05 (SD 0.07) mM for 50 and 87.9 g, respectively).

#### *Plasma metabolite responses with 89.7 g slowly hydrolysed starch in diabetic subjects*

Increasing the quantity of the S starch consumed to 89.7 g resulted in an increase in the maximum glucose change to 2.63 mM. This was lower than but not significantly different from that observed following 50 g R starch ( $P > 0.40$ ), and although numerically higher than that observed for the



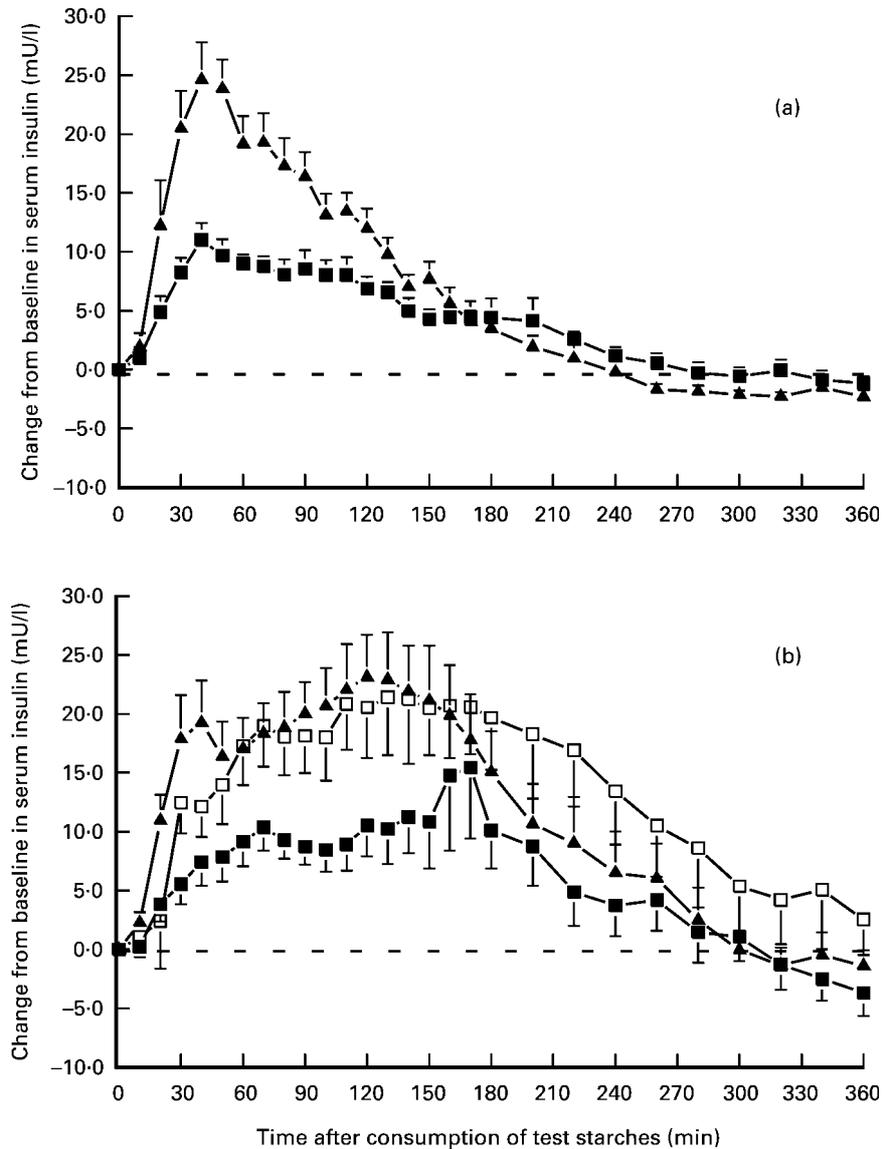
**Fig. 2.** (a), Change from baseline in plasma glucose concentrations after consumption of 50 g rapidly hydrolysed ( $\blacktriangle$ ) and 50 g slowly hydrolysed ( $\blacksquare$ ) starch for healthy subjects ( $n$  8); (b), change from baseline in plasma glucose concentrations after consumption of 50 g rapidly hydrolysed starch ( $\blacktriangle$ ;  $n$  13), 50 g slowly hydrolysed starch ( $\blacksquare$ ;  $n$  13) and a higher dose (89.7 g) of slowly hydrolysed starch ( $\square$ ;  $n$  11) for subjects with type 2 diabetes. Values are means, with standard errors of the mean represented by vertical bars. For details of subjects and procedures, see Table 1 and p. 854.

lower dose of the S starch, was not significantly different from this value ( $P=0.117$ ; Table 3). Consumption of 89.7 g S starch resulted in a similar decrease in plasma NEFA concentrations to that observed with 50 g S starch, but the nadir was achieved later, and was sustained throughout the study period. Plasma 3-hydroxybutyrate concentrations were also lower at the end of the study period than those observed after consuming 50 g S starch.

#### <sup>13</sup>C metabolism

<sup>13</sup>CO<sub>2</sub> output in breath (Fig. 4) was determined as a measure of oxidation of glucose absorbed from the R

starch, which was naturally enriched with <sup>13</sup>C. Breath <sup>13</sup>CO<sub>2</sub> output was similar for the healthy and type 2 diabetic subjects for about the first 230–270 min. Thereafter, rates of breath <sup>13</sup>CO<sub>2</sub> output fell for the healthy subjects whilst rates remained approximately constant for the diabetic subjects until very late in the study period. Because the background enrichment of breath <sup>13</sup>CO<sub>2</sub> was slightly lower for the diabetic than for the healthy subjects, apparent <sup>13</sup>C dose was higher for the former subjects (Table 4). There were no between-subject group differences in either the total <sup>13</sup>C recovered in breath or the percentage administered dose recovered up to 360 min after the test meal (Table 4).



**Fig. 3.** (a), Change from baseline in plasma insulin concentrations after consumption of 50 g rapidly hydrolysed (▲) and 50 g slowly hydrolysed (■) starch for healthy subjects ( $n=8$ ); (b), change from baseline in plasma insulin concentrations after consumption of 50 g rapidly hydrolysed starch (▲;  $n=13$ ), 50 g slowly hydrolysed starch (■;  $n=13$ ) and a higher dose (89.7 g) of slowly hydrolysed starch (□;  $n=11$ ) for subjects with type 2 diabetes. Values are means, with standard errors of the mean represented by vertical bars. For details of subjects and procedures, see Table 1 and p. 854.

#### Quantification of carbohydrate and lipid oxidation

From measurements of  $O_2$  consumption and  $CO_2$  production over the study period, cumulative rates of carbohydrate and lipid oxidation have been calculated and are shown in Table 5. Incremental changes in rates of carbohydrate oxidation above baseline and lipid oxidation below baseline values are shown in Fig. 5. Figs. 5 (a) and (b) show that the increase in carbohydrate oxidation after consuming the test starches was similar for the healthy subjects for both starches but was reduced in the diabetic subjects consuming the S starch compared with the R starch. The incremental area over the curve was 7.43 g/180 min for the R starch compared with 1.58 g/180 min for the S starch ( $P<0.05$ ). Lipid oxidation was reduced following the consumption of the test starches

(Figs. 5 (c) and (d)) and, although not statistically significant, was reduced more after the R starch compared with the S starch in both subject groups. Oxidation of exogenous carbohydrate, determined from indirect calorimetry and  $^{13}C$  output measurements after consumption of the R starch, was similar for both subject groups. However, when expressed as a percentage of total carbohydrate oxidation, exogenous carbohydrate oxidation was significantly lower for the diabetic subjects compared with the healthy subjects (10.0 v. 13.3%;  $P<0.05$ ).

#### Discussion

The rate and extent of starch digestion in the small intestine play important roles in determining glycaemic

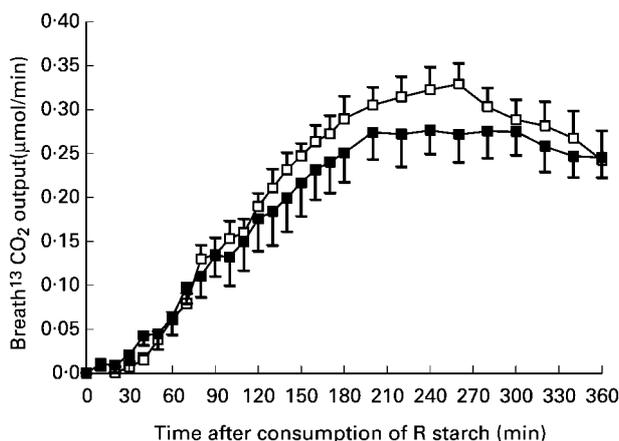
**Table 3.** Derived parameters of plasma glucose and insulin responses for subjects following consumption of test starches\* (Mean values and standard deviations)

	Healthy subjects				Diabetic subjects					
	50 g R starch (n 8)		50 g S starch (n 8)		50 g R starch (n 13)		50 g S starch (n 13)		89.7 g S starch (n 11)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Maximum glucose change (mm)	2.45 <sup>a</sup>	0.86	1.29 <sup>b</sup>	0.38	3.12 <sup>a</sup>	0.85	1.78 <sup>b,c</sup>	1.00	2.63 <sup>a,c</sup>	1.20
Time to glucose peak (min)	55 <sup>a</sup>	20.0	61 <sup>a</sup>	10.8	77 <sup>a</sup>	20.5	84 <sup>a</sup>	26.9	96 <sup>a</sup>	21.1
IAUC for glucose (mm × min)	205 <sup>a</sup>	85.9	134 <sup>a</sup>	62.7	366 <sup>a</sup>	99	191 <sup>b</sup>	132	302 <sup>a</sup>	157
IAUC for insulin (mU/l × min)	2192 <sup>a</sup>	488	1150 <sup>b</sup>	405	2859 <sup>a</sup>	1572	1640 <sup>a</sup>	1279	2334 <sup>a</sup>	1627
Minimal plasma NEFA concentration (mm)	0.02 <sup>a</sup>	0.03	0.08 <sup>b</sup>	0.12	0.12 <sup>a</sup>	0.07	0.18 <sup>a</sup>	0.15	0.15 <sup>a</sup>	0.16
Time to NEFA minimum (min)	93 <sup>a</sup>	29	103 <sup>a</sup>	17	138 <sup>a</sup>	18	146 <sup>a</sup>	53	170 <sup>a</sup>	38
IAOC, for NEFA (mm × min)	-92 <sup>a</sup>	42	-93 <sup>a</sup>	55	-85 <sup>a</sup>	21	-75 <sup>a</sup>	29	-87 <sup>a</sup>	46

R, rapidly hydrolysed; S, slowly hydrolysed; IAUC, incremental area under the curve; NEFA, non-esterified fatty acids; IAOC, incremental area over the curve.

<sup>a,b,c</sup>Mean values with unlike subscript letters within a row, within each subject group, were significantly different ( $P < 0.05$ ).

\* For details of subjects and procedures, see Table 1 and p. 854.



**Fig. 4.** Breath <sup>13</sup>CO<sub>2</sub> output after consumption of 50 g rapidly hydrolysed (R) starch for healthy subjects (□; n 8) and for subjects with type 2 diabetes (■; n 13). Values are means, with standard errors of the mean represented by vertical bars. For details of subjects and procedures, see Table 1 and p. 854.

**Table 4.** <sup>13</sup>C dose and recovery in breath carbon dioxide of subjects following consumption of 50 g rapidly hydrolysed starch\* (Mean values and standard deviations)

	Healthy subjects (n 8)		Diabetic subjects (n 13)	
	Mean	SD	Mean	SD
<sup>13</sup> C dose (µg above background)	3568 <sup>a</sup>	122	3758 <sup>b</sup>	214
<sup>13</sup> C recovered in breath (µg/360 min)	1000 <sup>a</sup>	209	957 <sup>a</sup>	259
Recovery of <sup>13</sup> C dose after 360 min (%)	28.1 <sup>a</sup>	5.9	25.4 <sup>a</sup>	6.7

<sup>a,b</sup>Mean values with unlike subscript letters within a row were significantly different ( $P < 0.05$ ).

\* For details of subjects and procedures, see Table 1 and p. 854.

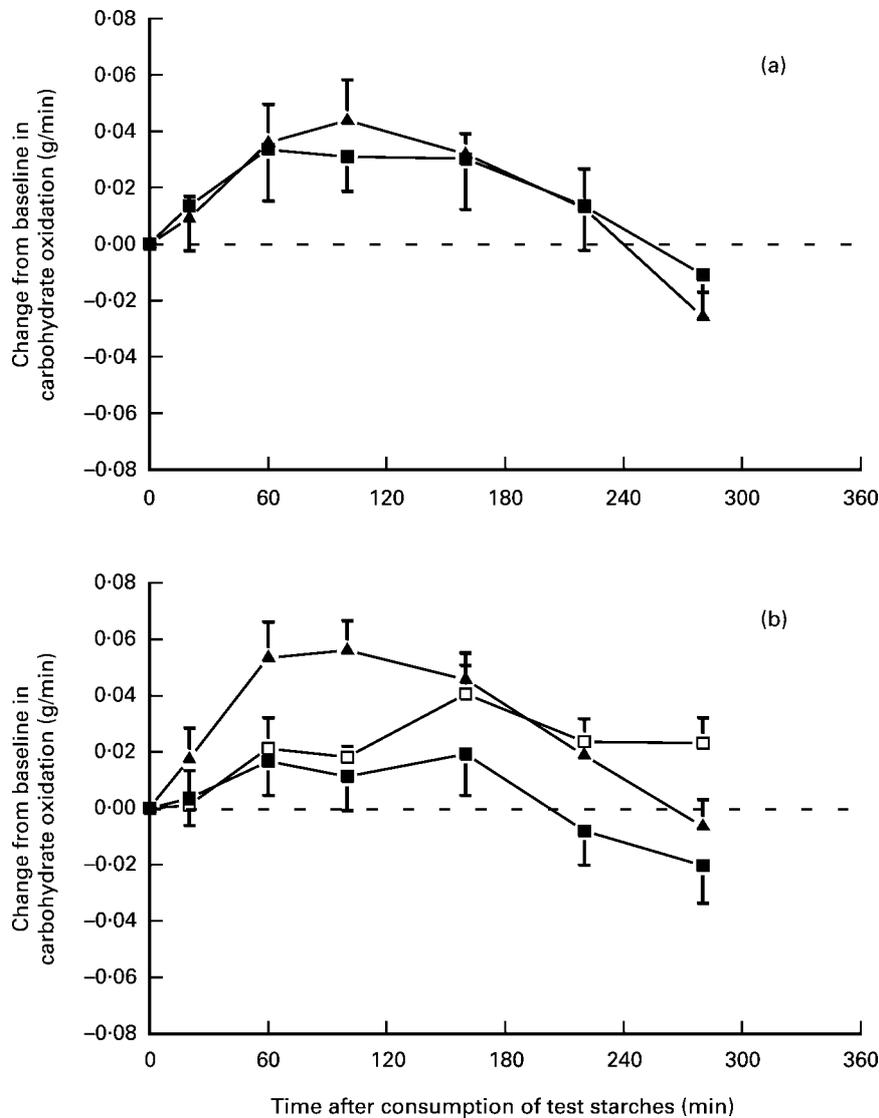
and insulinaemic responses following a meal and are affected by the source and degree of processing during food preparation (Food and Agriculture Organization/World Health Organization, 1998; Mathers & Daly, 2001). Several methods are available for the classification of starches, based both on their *in vitro* hydrolysis under a variety of incubation conditions and their digestion *in vivo*. For example, the extent of glucose release from starch hydrolysis under carefully defined conditions forms the basis of a current proposal for classification of starch fractions including rapidly digestible starch, slowly digestible starch and resistant starch (Englyst *et al.* 1992). These components are included with other dietary carbohydrate sources (free sugars and maltodextrins) when considering the potential availability of glucose from foods in the small intestine as either rapidly available glucose or slowly available glucose (Englyst *et al.* 1999). Diets which contain large amounts of rapidly digested carbohydrates result in elevated blood glucose and insulin responses, which are risk factors for increased insulin resistance and the development of type 2 diabetes (Salmerón *et al.* 1997a,b). In contrast, diets which contain large amounts of carbohydrates resistant to small-intestinal digestion and which deliver starch to the large bowel may protect against such conditions and also against other non-communicable diseases including colon cancer (Cassidy *et al.* 1994; Hylla *et al.* 1998).

The usefulness of *in vitro* data in predicting *in vivo* responses in human subjects remains uncertain. Englyst *et al.* (1999) have suggested that the assessment of rapidly available glucose using an *in vitro* hydrolysis procedure was a good indicator of blood glycaemic response. To date, probably the most extensively investigated method for predicting glucose response is the measurement of GI, and lists of GI exist for a wide range of foods (Foster-Powell & Brand-Miller, 1995). However, this requires experimentation with human volunteers and the

**Table 5.** Cumulative rates of carbohydrate and fat oxidation following consumption of test starches\* (Mean values and standard deviations)

	Healthy subjects				Diabetic subjects					
	50 g R starch (n 8)		50 g S starch (n 8)		50 g R starch (n 13)		50 g S starch (n 13)		89.7 g S starch (n 10)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total carbohydrate oxidation (g/180 min)	35.3 <sup>a</sup>	8.3	33.4 <sup>a</sup>	8.0	39.3 <sup>a</sup>	7.3	34.3 <sup>b</sup>	6.8	34.6 <sup>a,b</sup>	8.5
Total fat oxidation (g/180 min)	7.9 <sup>a</sup>	4.2	8.9 <sup>a</sup>	4.6	9.7 <sup>a</sup>	2.7	11.4 <sup>a</sup>	3.3	11.8 <sup>a</sup>	3.3

<sup>a,b</sup>Mean values with unlike subscript letters within a row, within each subject group, were significantly different ( $P < 0.05$ ).  
 \* For details of subjects and procedures, see Table 1 and p. 854.



**Fig. 5.** (a), Change from baseline in total carbohydrate oxidation rate assessed by indirect calorimetry data after consumption of 50 g rapidly hydrolysed (▲) and 50 g slowly hydrolysed (■) starch for healthy subjects (n 8); (b), change from baseline in total carbohydrate oxidation rate after consumption of 50 g rapidly hydrolysed starch (▲; n 13), 50 g slowly hydrolysed starch (■; n 13) and a higher dose (89.7 g) of slowly hydrolysed starch (□; n 11) for subjects with type 2 diabetes; (c), change from baseline in total fat oxidation rate after consumption of 50 g rapidly hydrolysed (▲) and 50 g slowly hydrolysed (■) starch for healthy subjects (n 8); (d), change from baseline in total fat oxidation rate after consumption of 50 g rapidly hydrolysed starch (▲; n 13), 50 g slowly hydrolysed starch (■; n 13) and a higher dose (89.7 g) of slowly hydrolysed starch (□; n 11) for subjects with type 2 diabetes. Values are means, with standard errors of the mean represented by vertical bars. For details of subjects and procedures, see Table 1 and p. 854.

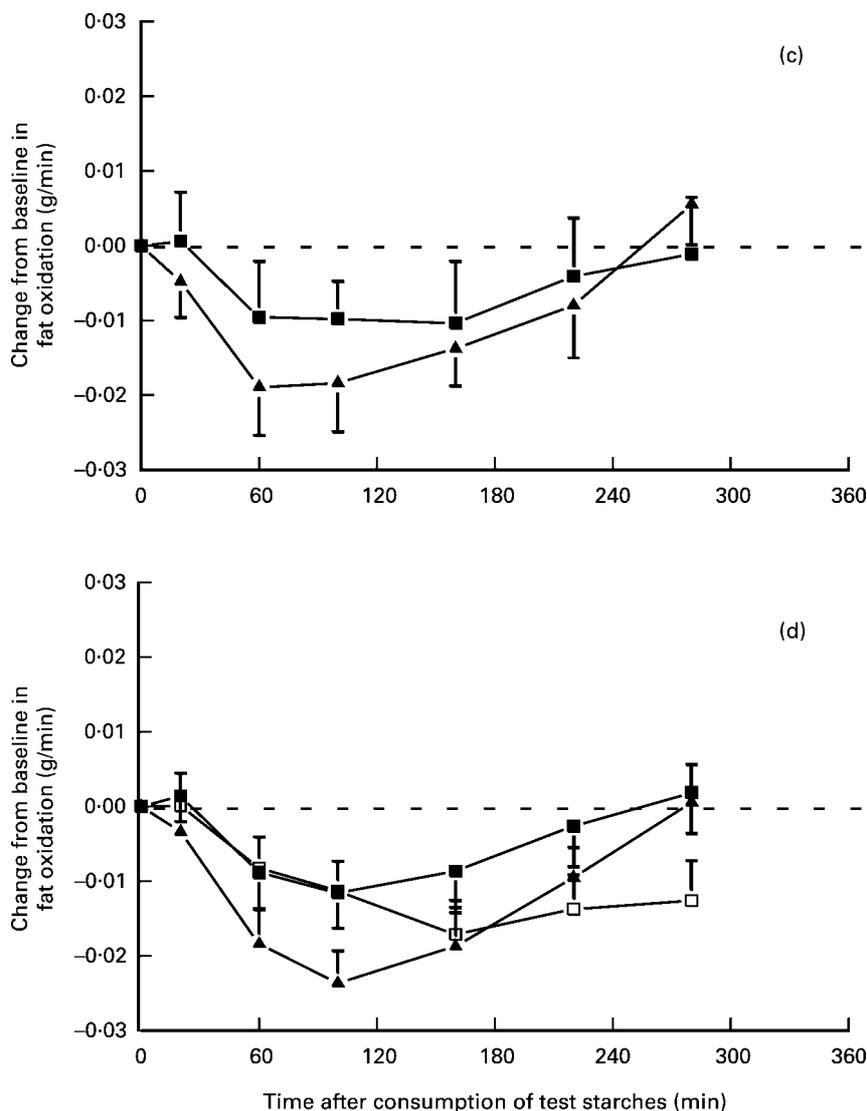


Fig. 5. Continued

applicability of the GI approach to complex meals and diets and across different population groups is debatable. The authors of the present study have adopted a simple *in vitro* hydrolysis procedure to predict the release of glucose from starch products. Whilst it has yet to be proven that this procedure can predict *in vivo* rates of digestion of complex food mixtures, the results clearly demonstrate that R and S starches have distinctly different patterns of hydrolysis *in vitro* with characteristically different patterns of postprandial glycaemia and insulinaemia when consumed in isolation as a single meal. The ratio of glucose released after 180 min *in vitro* for R starch:S starch was approximately 1.8:1 (Fig. 1), which compares with a ratio of 1.5:1 and 1.9:1 for the 180 min IAUC or 1.9:1 and 1.8:1 for the maximum glucose change in the healthy and type 2 volunteers, respectively (Table 3) as measures of short-term glycaemic response *in vivo*. These data suggest that the *in vitro* assay had good discriminatory powers and has potential as a rapid screening method for

the evaluation of different starch products. However, the behaviour of the starches when combined in a mixed meal or incorporated into a processed food has yet to be investigated.

The ratio of the IAUC for R starch:S starch in the diabetic subjects indicated that the more rapidly digested starch produced nearly 80% greater postprandial glycaemic response. Increasing the dose of the R starch to 89.7 g tested the hypothesis that providing 80% more of the slowly hydrolysed starch in the test drink would produce a plasma glucose response equal to that for the standard dose (50 g) of the R starch. While it was clear that the bigger dose produced a greater response, the pattern of change in blood glucose was quite different from that with the R starch. Peak glucose concentration was achieved much later and plasma glucose concentration remained elevated above fasting levels for longer. Although none of the outcome measures with the higher dose of the S starch in the third experimental period was

statistically different from that for the R starch, it is unlikely that the higher dose of the S starch is metabolically equivalent to the standard dose of the R starch in type 2 diabetics. The smaller than predicted plasma glucose response with the higher dose of the S starch is consistent with observations of a linear glycaemic dose–response for intermediate GI foods delivering up to 50 g available carbohydrate with a flattening of the response for doses between 50 and 100 g (Wolever *et al.* 1991).

The measurement of expired  $^{13}\text{CO}_2$  has been used to determine carbohydrate, lipid and amino acid oxidation using both invasive infusion procedures and after the oral consumption of substrates labelled with  $^{13}\text{C}$  (Seal, 1997). In many studies, an exogenous label is used to enrich the substrate pool (for example, see Daly *et al.* 2000), but improved analytical techniques have resulted in the increased use of substrates naturally enriched with  $^{13}\text{C}$ . Maize starch is naturally enriched due to the discriminatory accumulation of the  $^{13}\text{C}$  molecule during  $\text{CO}_2$  fixation in the C4-pathway for photosynthesis in the maize plant (Hatch & Slack, 1966). Other C-containing biomolecules (including protein) in the maize plant will similarly be enriched following the redistribution of fixed  $^{13}\text{C}$ . However, in the present experiment, the contribution of these components to  $^{13}\text{CO}_2$  output will be negligible due to the high purity of the test starches. [ $^{13}\text{C}$ ]Glucose released during the hydrolysis of maize starch is absorbed and its subsequent oxidation results in the production of  $^{13}\text{CO}_2$  in breath. Measurement of the appearance of labelled glucose in blood shows that the pattern of [ $^{13}\text{C}$ ]glucose in blood is very different to the glycaemic response determined by the measurement of total blood glucose (Normand *et al.* 1992). Measuring breath  $^{13}\text{CO}_2$  output compares with measurements of the appearance of [ $^{13}\text{C}$ ]glucose in blood (Normand *et al.* 1992), and can, therefore, be used to estimate rates of glucose absorption and oxidation. The data of Normand *et al.* (1992), and the results from the present study, show that glucose absorption following a starchy meal continues well after the insulin response has resulted in the return of blood glucose concentrations to fasting levels and that oxidation of the absorbed glucose continues for several hours after the meal. Daly *et al.* (2000) have also used this method to quantify rates of carbohydrate oxidation using uniformly labelled [ $^{13}\text{C}$ ]fructose and [ $^{13}\text{C}$ ]glucose and demonstrated different rates of oxidation of the two sugars following high-starch and high-sucrose meals. It is probable that the pattern of rate of  $^{13}\text{CO}_2$  output is closely related to the pattern of uptake of glucose from the gut, albeit delayed whilst the postprandial insulin surge shuts down lipolysis and cells switch to glucose as the main oxidizable fuel. The sustained use of glucose as the oxidizable fuel is also confirmed by the lower circulating concentrations of 3-hydroxybutyrate and NEFA at the end of the study period following consumption of the S starch, especially after the higher dose consumed in the third experimental period by the diabetic subjects. For the R starch, the rate of  $^{13}\text{CO}_2$  output was lower for the diabetics than for the healthy subjects until 280 min after which time the rate of output fell faster for the healthy subjects. This may be a consequence of the reduced insulin sensitivity in

diabetics which delayed the entry of glucose into cells and led to the slightly lower recovery of administered  $^{13}\text{C}$  as  $^{13}\text{CO}_2$ .

For the R starch only, approximately 5–7% of the  $^{13}\text{C}$  dose was recovered after 180 min and this increased at a linear rate, reaching 24–28% by the end of the 6 h study period, confirming the prolonged metabolism of the labelled exogenous glucose long after the plasma glycaemic response had ended. Over a slightly longer time period of 8 h, Achour *et al.* (1997) recovered approximately 30% of  $^{13}\text{C}$  from a 50 g dose of naturally-enriched maize starches in breath. This group also showed that the recovery of  $^{13}\text{C}$  was lower for a partially indigestible (retrograded) maize starch compared with a digestible (pre-gelatinized) product. Their study confirms the usefulness of the  $^{13}\text{C}$  methodology in investigating the metabolic effects of consuming modified starches in addition to the observations for raw products described in the present experiment.

The long-term benefits of reducing the rate of glucose absorption with the use of slowly digested starches (expected to have lower GI) include greater glycaemic control in subjects with diabetes or metabolic syndrome and lowered NEFA concentrations in patients with hypertriglycerolaemia (Food and Agriculture Organization/World Health Organization, 1998; Wolever, 2000). There may also be benefits in healthy individuals by reducing postprandial fatty acids concentrations (Wolever *et al.* 1995) and in preventing the development of type 2 diabetes and cardiovascular disease (Salmerón *et al.* 1997a,b; Frost *et al.* 1998, 1999). The results from the present study show that starches with contrasting hydrolysis characteristics *in vitro* have correspondingly different postprandial glycaemic and insulinaemic responses in both healthy volunteers and type 2 diabetic subjects. Based on the appearance of  $^{13}\text{CO}_2$  in breath, there was strong evidence that the oxidation of exogenous glucose continued at high rates long after the plasma glycaemia had returned to fasting levels. These observations reinforce the utility of  $^{13}\text{CO}_2$  tracking as a simple quantitative procedure for characterizing the rate and extent of starch digestion and glucose disposal through oxidation.

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