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ORIGINAL CONTRIBUTION

Glyceamic and insulinaemic response to mashed potato alone, or with broccoli, broccoli fibre or cellulose in healthy adults

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Abstract
Purpose To examine the role of realistic serving sizes of broccoli, broccoli fibre and cellulose co-consumed with mash potato, or mashed potato eaten alone, on glycaemic and insulinaemic responses (GR and IR) in healthy adults.
Method A non-blind randomized crossover trial was conducted with thirteen healthy subjects consuming four different meals. Capillary blood samples between 0 and 180 min were analysed for glucose and insulin. The incremental area under the fasting blood glucose and insulin curves (iAUC) was calculated for different time increments. Differences in GR and IR between meals were assessed by repeated measures analysis of variance.
Results The immediate GR and IR to one serving of mashed potato eaten with two servings of broccoli were significantly lower than mashed potato eaten alone. The peak, incremental peak and $iAUC_{0–30min}$ for GR and $iAUC_{0–30min}$ for IR were all significantly lower for the broccoli–potato meal. This meal also takes longer to return to fasting baseline with a time-delayed lag in IR and GR compared to the potato only meal. The $iAUC_{60–120min}$ for IR was significantly greater for the broccoli–potato meal compared to the other meals. Yet there was no corresponding significant difference between the broccoli–potato meal and the other meals for peak, incremental peak IR or any other iAUCs for GR and IR. For the potato meals containing added broccoli fibre or cellulose, no significant differences in GR or IR were observed when compared with the potato eaten alone.
Conclusion Co-consumption of cooked broccoli with mashed potato has a significant effect on glycaemic and insulinaemic responses compared to potato eaten alone. Our study suggests broccoli eaten with potato improves glucose homeostasis and therefore indicates a general beneficial nutritional role for broccoli when eaten with a carbohydrate staple.

Keywords Blood sugar · Vegetable · Starch · Meal · Carbohydrate staple

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Introduction

Sustained postprandial hyperglycaemia and hyperinsulinaemia in healthy adults are strongly associated with an increase in the risk of developing type 2 diabetes and/or metabolic syndrome. The postprandial glycaemic (GR) and insulinaemic responses (IR) of starch rich-carbohydrate staples, such as bread, rice, pasta and potato, and the factors that might modulate them, have therefore received extensive attention [1]. This includes understanding the effect of adding additional ingredients to carbohydrate staples and the effect of eating them as part of a mixed meal. Other important variables include the amount and type of glycaemic carbohydrate consumed, and the regime of processing during food preparation. The general glycaemic and insulinaemic effects in healthy adults of protein, fat, dietary fibre and organic acids additions to carbohydrate staples are all widely investigated [1]. Many studies have assessed additions of either purified or cereal-derived

dietary fibre. Far fewer studies have examined the effects of co-consumed vegetables on GR/IR in healthy adults even though the many meals containing carbohydrate staples, such as cooked potatoes and rice, include them.

Studies on co-consumed vegetable–staple carbohydrate combinations would enable one to assess whether vegetable has any direct effect on the GR/IR of a carbohydrate staple. Yet there is surprisingly very little research on this topic. A 120 g serving of fresh salad (cucumber, tomato and lettuce) consumed together with just over two portions of warm mashed potato (363 g) had no significant effect ($P > 0.05$) on GR/IR in healthy subjects compared to that for an equivalent serving of mashed potatoes eaten alone [2]. In another study, 120 g boiled Chinese cabbage (bok choy) and 200 g boiled rice were eaten by healthy subjects [3]. The GR, as assessed by the incremental area above the fasting blood glucose curve over 120 min ($iAUC_{0–120min}$) and peak glucose, was moderately, but still significantly ($P < 0.05$) less, compared to 200 g of rice eaten alone [3]. There was also a significant difference in peak insulin response but not for $iAUC_{0–120min}$ for IR between these two meals [3].

There are some further studies with mixed meals. Healthy adults served a typical Swedish lunch (creamed potatoes, bread, meatballs, lingonberry jam and light beer) with or without carrots, spinach, peas or Brussels sprouts, containing 4.4 g of dietary fibre, all had similar GR or IR ($P > 0.05$), except for the meal with added spinach [4]. For the spinach meal, a significantly lower ($P < 0.05$) postprandial IR (peak and $iAUC_{0–120min}$) was observed [4]. In a follow-up study, where the dose of spinach was increased to 250 g, containing 7.3 g dietary fibre, postprandial GR, in addition, was also significantly less [5] than the control meal without spinach. At a more realistic serving size of vegetables, the postprandial GR was lower when 60 g each of boiled broccoli and spinach containing 4.4 g dietary fibre were added to a complex Japanese mixed meal, compared with the same meal without vegetables [6].

In most of the above-mentioned studies, a thorough discussion of the possible mechanisms behind a response in GR/IR is often lacking. Quite often discussion of the GR/IR of vegetable on carbohydrate rich staples in mixed meals is overshadowed by discussion of the effects of other protein and fat-rich meal components [2, 3].

In a rat study with minimally processed broccoli fibre added to corn oil, it was shown that physical properties of broccoli fibre in the small intestine may reduce enterohepatic bile acid recycling and intestinal lipid absorption with a consequent increase in faecal bile acid excretion [7]. Indeed, the physical properties of digested plant matter and its role in glycaemic health has recently received renewed attention and possible mechanisms are currently under renewed debate [8, 9]. There are also other factors of potential importance. This includes, among others, type of vegetable and how its preparation, vegetable-to-carbohydrate staple ratio, and quantity and composition of other food components and nutrients. It is clear further work is required to understand the role of vegetables and the mechanisms responsible for their potential glycaemic and insulinaemic health effects. Clinical in vivo feeding experiments with humans should play a key part of this work.

In our current study, we have selected mashed potato as the carbohydrate staple to be consumed by healthy human subjects at a typical and representative serving size of 150 g. To this, we have assessed the effect of adding two servings of boiled broccoli florets on postprandial GR and IR. Minimally processed broccoli fibre was also added to mashed potato to investigate a possible role of dietary fibre and cell wall structure. This portion of broccoli fibre had an equivalent content of total fibre to that found in two servings of broccoli. An equivalent amount of cellulose to the broccoli fibre preparation was also added to mashed potato as a third meal combination to act as an insoluble dietary fibre-rich negative control. Cellulose was chosen because previous clinical studies have validated it as a suitable negative control when investigating the GR lowering effect of soluble fibres [10].

Our experimental design takes inspiration from the studies discussed above that show cooked cruciferous vegetables, such as broccoli, to have potential positive effects on lowering GR/IR of a co-consumed carbohydrate staple. Secondly, that the physical bulk properties of the vegetable matter in the small intestine might, in part, explain this effect. The meals have also been prepared and processed in a way that is representative of the situation for a typical ‘ready-to-eat’ meal available from the supermarket apart from the test meals we have made were subjected to two, rather than the usual one, cooking–cold storage (>2 days) cycle prior to re-heating and immediate consumption.

Materials and methods

Study meals

The meals were prepared in a professional kitchen used for product development of ready meals (Fjordland AS, Oslo, Norway). Each contained 150 g mashed potato. To ensure homogeneity, the mashed potato was prepared in one large batch. Commercial chilled–stored (>2 days) sous-vide cooked potatoes that were pre-blanched (Hoff AS, Jæren, Norway) of the variety Faxe were pressed through a potato ricer and mixed by hand. Broccoli var., Iron Man, was obtained via Bama AS from Skallerød farm, Jeløy, Norway and cold–stored at 2 °C in the dark until use. Floret bouquets were cut from the broccoli heads, washed in tap

water, cooked in a pan of boiling water until tender, and then cooled in cold water. Broccoli florets of 180 g portions were immediately vacuum packed. Equivalent portions (150 g) of potato alone, potato mixed with 8 g milled broccoli fibre or 8 g milled cellulose were also identically vacuum packed at the same time. All packed meals received heat treatment in a Convotherm combi-steamer for 30 min at 98 °C. They were then cooled in running cold water for 20 min before chilled overnight transport to Leatherhead, UK. Prior to consumption, each meal was re-heated in its vacuum bag for 6 min in boiling water apart from the broccoli which was re-heated for 11 min. The 'ready-to-eat' core-meal temperature was 70–72 °C. Apart from the broccoli–potato combination, a measured glass of water was supplied for consumption with the meal to ensure a total consumed meal mass of 330 g throughout the study.

The nutrient composition of the meals was analysed as follows. Protein was estimated (N × 6.25) from the analysis of N by the method of Kjeldahl [11]. Fat was determined gravimetrically following acid hydrolysis, extraction into diethyl ether and petroleum ether and evaporation [12]. Total dietary fibre was determined gravimetrically according to AOAC 985.28 [13]. Total non-starch polysaccharide in dried minimally processed broccoli fibre and in the cooked broccoli was determined by the method of Englyst [14] via GC-FID analysis of alditol acetates. Sugars were determined as the sum of sucrose, glucose and fructose after extraction in 50 % water:methanol by anion-exchange chromatography with pulsed amperometric detection [15]. Total and resistant starch 'as eaten' was determined by AOAC 2002.02 within 1 h of re-heating the potato. Available CHO was subsequently calculated as described by Brouns et al. [16]. Water content was determined gravimetrically following drying at 103 °C to constant weight [17]. Ash was determined as the inorganic residue remaining after removal of all water and organic matter by heating at 550 °C [18]. Total energy content was calculated according to EU Council Directive 1169/2011 [19]. The nutrient composition of the test foods is shown in Table 1.

Preparation of broccoli fibre

A similar milling and cold-water extraction scheme described by Mandimika et al. [7] for stalk-derived broccoli fibre was employed. Broccoli (33 kg, 10–15 cm stalk and with head) were washed in tap water. Thereafter, the stalk was cutoff from the head and discarded to yield 23.3 kg floret bouquets. These were then cut up in a Garant MTK 661 tabletop bowl rotary chopper and yielded 21 kg of about 2- to 5-mm broccoli floret particles. These were further reduced in size, water added, and the resultant solid–liquid suspension fed as a slurry into a Fryma MZ-80 toothed colloid mill with a rotor–stator setting of

Table 1 Nutrient composition of the test meals

(g/portion)	Meal size[a]	Meal size[b]	ACHO	Digestible starch	Resistant starch	Sugars	Total fibre	Fat	Protein	Ash	Water[a]	Water[b]	Energy (kcal)
Mashed potato	150	330	22.2	19.5	1.7	0.8	2.9	0.6	2.7	0.9	120	300	111
Mashed potato and broccoli	330	330	24.0	19.5	1.7	2.5	8.1	1.8	7.8	1.9	285	285	160
Mashed potato and broccoli fibre	158	330	22.2	19.5	1.7	0.8	7.8	0.6	4.6	1.1	122	294	127
Mashed potato and cellulose	158	330	22.2	19.5	1.7	0.8	11	0.6	2.7	0.9	120	292	124

[a] Not including glass of water consumed with the meal

[b] Including glass of water consumed with the meal

0.5 mm. A nylon 250 μm sieve mesh (2 × 1 m) was used to enclose the slurry of broccoli floret fragments, and these were washed in running tap water (approx. 8 °C) overnight. Using a 20 L Speidel hydropress (1–2 bar pressure), water held in the washed floret fragments was pressed-out and discarded. The resultant fibrous cake was thinly spread on baking paper, placed on a tray, and dried to constant weight in an oven at 65 °C. The yield was 1 kg. About 300 g of this material was then dry milled into a fine powder with a Retsch hammer mill fitted with a 1 mm sieve. Particle size distribution was determined with a Sympatec HELOS laser diffraction sensor with ROSOS dry dispersing unit fitted with a R6 lens (0.5–1750 μm).

Preparation of cellulose

Cellulose sheets (28.5 × 17.5 cm) manufactured as a substrate to make food grade chemically modified cellulose were cut into 10 × 2 mm pieces with scissors. These pieces were dry milled into a fine powder with a Retsch hammer mill fitted with a 1-mm sieve. Particle size distribution was determined with a Sympatec HELOS laser diffraction sensor with ROSOS dry dispersing unit fitted with a R6 lens (0.5–1750 μm).

Scanning electron microscopy

The sample was mounted on an aluminium stub using double-sided tape coated with carbon. The sample was then coated with gold/palladium using a SC7640 auto/manual high-resolution sputter coater (Quorum Technologies, Ashford, UK). An EVO-50-EP environmental scanning electron microscope (Zeiss, Cambridge, UK) was used to study the sample at a magnification of x2000.

Subjects

Volunteers were pre-screened and asked initial recruitment questions in order to determine their suitability to take part in the study. The nature of the study and their involvement and responsibilities were described to them. Eligible volunteers that were willing to participate were presented with an information sheet, containing study details, along with a written consent form at least 3 days before starting the study. The inclusion criteria were age: 18–65 years, gender: male or female, BMI 18–27 kg/m^2, self-diagnosis as healthy at the time of recruitment confirmed by medical questionnaire. Fasting blood glucose: 4–6 mmol/L. Subjects were excluded from the study if they had any history of diabetes or had consumed anything apart from water 12 h prior to starting the test.

Fifteen healthy subjects were recruited for one single cohort. Two subjects did not consume all test meals in the allotted 15 min and were thus excluded from further analysis. Thirteen subjects completed the study of which 12 were female. The mean age of these subjects was 47.6 (SEM 3.29) years with a mean BMI of 22.6 (SEM 0.53) kg/m^2. The study was conducted according to guidelines laid down in the Declaration of Helsinki and study design approved by the West Kent Research Ethics Committee, Aylesford, UK. Written informed consent was obtained from all subjects. All clinical testing was conducted at Leatherhead Food Research, UK in October 2014.

Study protocol

The night before the test the subjects were instructed to avoid strenuous physical activity, refrain from smoking or consuming alcohol the evening before a test or during the day of the test. The subjects were instructed to consume a similar carbohydrate-based evening meal before each test session. Subjects were also instructed to fast from 20:00 the night before a test. Water consumption was not restricted. Subjects should not have had a similar test for the last 48 h (washout time). On each test day, the volunteers arrived at the Human Nutrition Unit, having fasted for at least 12 h prior to commencement, and they were seated and asked to remain so for the duration of the test. Upon arrival their blood glucose levels were checked using a hand-held glucometer to ensure they had fasted correctly and were suitable to take part. Once each subject was relaxed and comfortable, they were asked to provide a baseline glucose measurement for that day, against which all of that day's subsequent assessments were measured. The subjects were given the four different potato-based meals in a non-blind randomized order on separate days (crossover) with a least 48-h washout between testing. Each subject presented with a study meal including a glass of water (were appropriate) was instructed to consume the whole amount within a 15-min period. The first blood sample was collected exactly 15 min after the first bite of the sample food. After this point, blood samples were taken at 15-min intervals for the first hour, 30-min intervals for the second hour and then after a 1-h interval for the third hour. Samples were collected at 0, 15, 30, 45, 60, 90, 120, 180 min.

Capillary blood samples were collected into small tubes containing lithium–heparin following a finger-prick, and centrifuged at 3000 rpm for 10 min to separate the plasma. The plasma samples were then analysed for glucose by an YSI 2300 Stat Plus Glucose and Lactate analyser. The sensitivity of the analyser is 0–50 mmol/L, and the margin of error is ±2 % or 0.2 mmol/L. Insulin was analysed in plasma using a sandwich ELISA (Mercodia, Uppsala, Sweden) according to manufactures instructions. Prior to insulin analyses, all plasma samples were stored at −80 °C.

Calculations and statistical analysis

The incremental area under the glucose and insulin response curves (iAUC) above baseline was calculated for specified time intervals between 0–180 min using the standard trapezoid geometric method [16]. Minitab version 17 was used for all statistical analysis. The Kolmogorov–Smirnov test revealed all data were normally distributed. Statistical differences between meals (fixed factor) were therefore assessed for subjects (random factor) by repeated measures ANOVA. The criterion for significance was a two-tailed $P < 0.05$. Comparison between meals was made with the post hoc Bonferroni pairwise test at a confidence interval of 95 %.

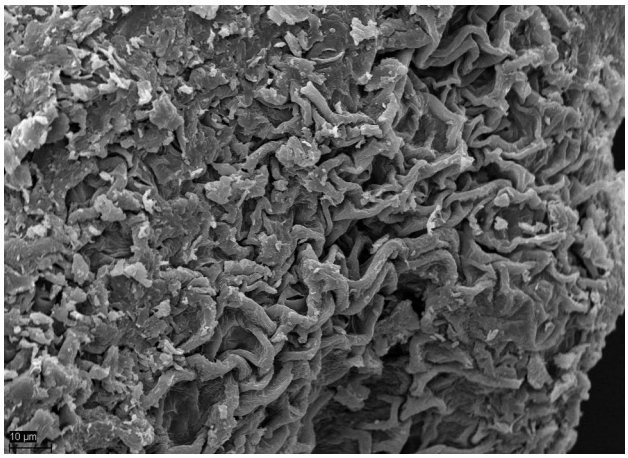

Fig. 1 Scanning electron microscope picture of the broccoli fibre. Magnification ×2000. *Scale bar* 10 µm

Results

All test meals were rich in available carbohydrate (22.2–24 g) almost exclusively originating from the potato. An extra 1.8 g available carbohydrate in the broccoli meal came from sugars in the broccoli (Table 1). All meals were low in protein and fat but quite high (7.3–11 g) in dietary fibre, apart from the potato only based meal that only had 2.7 g dietary fibre (Table 1). All meals had a low-energy content (Table 1). They also had an equal mass when co-consumed with a glass of water (Table 1). The 'liquid fraction' (glass of water + moisture in the meal food components) was therefore similar in all meals (285–300 g). Scanning electron microscopy revealed the broccoli fibre to comprise mostly collapsed plant cell walls (Fig. 1) particularly rich in polysaccharide but also protein (Fig. 1; Table 1). The dietary fibre and NSP content and NSP composition in the broccoli and in the minimally processed broccoli fibre were identical when expressed in terms of amounts in the respective meals (Table 2). The cumulative size distribution of 10–90 % of dehydrated broccoli fibre particles was 45–430 µm. The size distribution of the cellulose was similar at 25–375 µm.

Table 3 and Fig. 2 show the postprandial GR and IR for all test meals. For each corresponding meal, both these physiological responses closely mirror one another (Fig. 2). Peak responses for all meals occurred at 30 min. For the broccoli–mashed potato meal, however, peak ($P = 0.009$), incremental peak ($P = 0.026$) and $iAUC_{0–30min}$ ($P = 0.003$) for GR were all significantly less compared to either potato consumed alone or potato mixed with cellulose (Fig. 2a; Table 3A). A similar trend was observed for the $iAUC_{0–30\ min}$ IR which was significantly lower ($P = 0.003$) for the broccoli–potato meal than the potato meal eaten alone.

Another difference is that the broccoli–potato meal takes 180 min to return to fasting baseline for both IR and GR (Fig. 2). There appears to be a time-delayed lag in IR and GR when compared to the other meals that returned to fasting baseline by 120 min (Fig. 2). This delayed response is nicely illustrated for $iAUC_{60–120min}$ for IR which was significantly greater ($P = 0.004$) compared to all other meals (Table 3B; Fig. 2b). Nevertheless, there was no corresponding significant difference between the broccoli–potato meal and the other meals for peak, incremental peak IR or any other iAUCs for GR or IR (Table 3; Fig. 2).

For mashed potato mixed and eaten with broccoli fibre, there is also no significant difference in GR or IR for peak, incremental peak or $iAUC_{0–30min}$, compared to the potato only and potato–cellulose meals (Table 3; Fig. 2). Apart from $iAUC_{0–30\ min}$, there was also no significant difference in these same parameters between the broccoli fibre–potato meal and the broccoli–potato meal (Table 3B). For the potato–cellulose meal, there was no significant difference in incremental peak glucose or $iAUC_{0–30min}$ for IR, compared to the broccoli–potato meal (Table 3). Otherwise, the cellulose–potato meal had a quite similar GR and IR to the potato only meal (Table 3; Fig. 2).

Discussion

Co-consumption of two servings of cooked broccoli with one serving of mashed potato appears to lower the immediate glycaemic impact and significantly reduce the resulting acute insulin demand in healthy subjects. Broccoli consumed with potato seems it might significantly suppress the rate of glucose loading and the release of stored insulin in response to the glucose challenge. Eaten with potato, broccoli serves to consequently delay and then elevate and extend the duration of the IR post-peak over fasting baseline. Nonetheless, while the GR, following broccoli addition to potato, was also delayed and extended post-peak,

Table 2 Dietary fibre and non-starch polysaccharides in broccoli and broccoli fibre

(g/meal portion)	Total fibre	Total NSP	Uronic acid	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
Broccoli	5.2	4.1	1.0	0.1	0.03	0.6	0.2	0.1	0.5	1.4
Broccoli fibre[A]	4.9	4.0	1.0	0.1	0.03	0.6	0.2	0.1	0.4	1.4

Values for carbohydrates all expressed as polysaccharide equivalents

[A] Values are for material prior to mixing with potato, subsequent packaging and heat treatment

Table 3 Fasting, peak, incremental peak and incremental areas under the curves above baseline for different periods between 0 and 180 min of capillary blood glucose (A) and insulin (B) in healthy subjects following the study meals (mean of $n = 13$, ±SE)

	Mashed potato	Mashed potato and broccoli	Mashed potato and broccoli fibre	Mashed potato and cellulose
(A)				
Concentration (mmol/l)				
Fasting	5.0 ± 0.1^{A}	5.1 ± 0.1^{A}	5.1 ± 0.1^{A}	5.1 ± 0.1^{A}
Peak	8.3 ± 0.3^{A}	7.5 ± 0.3^{B}	7.9 ± 0.3^{AB}	8.2 ± 0.3^{A}
Incremental peak	3.2 ± 0.3^{A}	2.5 ± 0.3^{B}	2.8 ± 0.3^{AB}	3.1 ± 0.3^{AB}
iAUC (mmol × min/L)				
0–30 min	55.3 ± 4.5^{A}	40.8 ± 3.8^{B}	47.8 ± 3.4^{AB}	54.9 ± 3.6^{A}
0–60 min	109.5 ± 10.4^{A}	98.0 ± 9.9^{A}	102.6 ± 9.3^{A}	110.6 ± 11.7^{A}
0–120 min	126.8 ± 16.6^{A}	129.9 ± 13.7^{A}	122.8 ± 13.8^{A}	128.2 ± 17.4^{A}
60–120 min	17.4 ± 7.5^{A}	31.9 ± 6.4^{A}	18.8 ± 5.7^{A}	17.6 ± 7.1^{A}
120–180 min	1.68 ± 1.3^{A}	4.8 ± 2.0^{A}	1.8 ± 0.7^{A}	1.7 ± 1.2^{A}
(B)				
Concentration (mU/l)				
Fasting	3.8 ± 0.6^{A}	5.3 ± 1.0^{A}	3.7 ± 0.5^{A}	5.1 ± 0.7^{A}
Peak	35.6 ± 3.6^{A}	31.2 ± 3.2^{A}	35.4 ± 2.8^{A}	32.3 ± 2.5^{A}
Incremental peak	31.8 ± 3.4^{A}	25.9 ± 3.1^{A}	31.7 ± 2.8^{A}	27.3 ± 2.6^{A}
iAUC (mU × min/L)				
0–30 min	$605.5 \pm 72.8.^{A}$	374.3 ± 50.9^{B}	543.7 ± 49.6^{A}	525.3 ± 51.55^{AB}
0–60 min	1109.8 ± 112.6^{A}	932.7 ± 123.4^{A}	1138.4 ± 89.0^{A}	1028.6 ± 102.2^{A}
0–120 min	1354.2 ± 134.3^{A}	1358.4 ± 179.5^{A}	1380.8 ± 114.3^{A}	1215.3 ± 129.5^{A}
60–120 min	244.4 ± 70.8^{A}	425.7 ± 86.3^{B}	242.5 ± 54.4^{A}	186.7 ± 64.5^{A}
120–180 min	50.4 ± 21.4^{A}	56.4 ± 26.6^{A}	34.1 ± 13.0^{A}	39.0 ± 21.2^{A}

Meals that share a letter are not significantly different

it was not elevated over that of the other meals subjected to significantly greater acute insulin responses. The broccoli evens out the homeostatic response of the carbohydrate load in the potato with the associated potential beneficial health implications.

A similar shift of the blood glucose profile was not observed when cooked bok choy or fresh salad was, respectively, eaten with rice or mashed potato compared to the carbohydrate staples eaten alone [2, 3]. Peculiarly, for the bok choy–rice study, the peak glucose response for rice eaten alone occurred at 60 min [3]. This unusual result is at odds with similar studies, even with Asian subjects, where peak postprandial GR for rice and other carbohydrate staples occurs at around 30 min [6].

The available carbohydrate load in our study was half that of most previous studies, and the vegetable portion was about one third more. The meal composition of two servings of broccoli to one serving of mashed potato would therefore maximize any contribution; the vegetable may have on the GR/IR of the mashed potato-based meal. Such a carbohydrate staple-to-vegetable ratio may represent a threshold where the effects of co-ingested vegetable start to exert its effects on GR/IR. This reinforces the conclusions of other studies that high doses of vegetable, preferably cooked, are required to have an effect on GR [5]. It seems the vegetable-to-carbohydrate serving size ratio is also very important, particularly, in a meal with low fat and protein content. Selection of the type of vegetable and

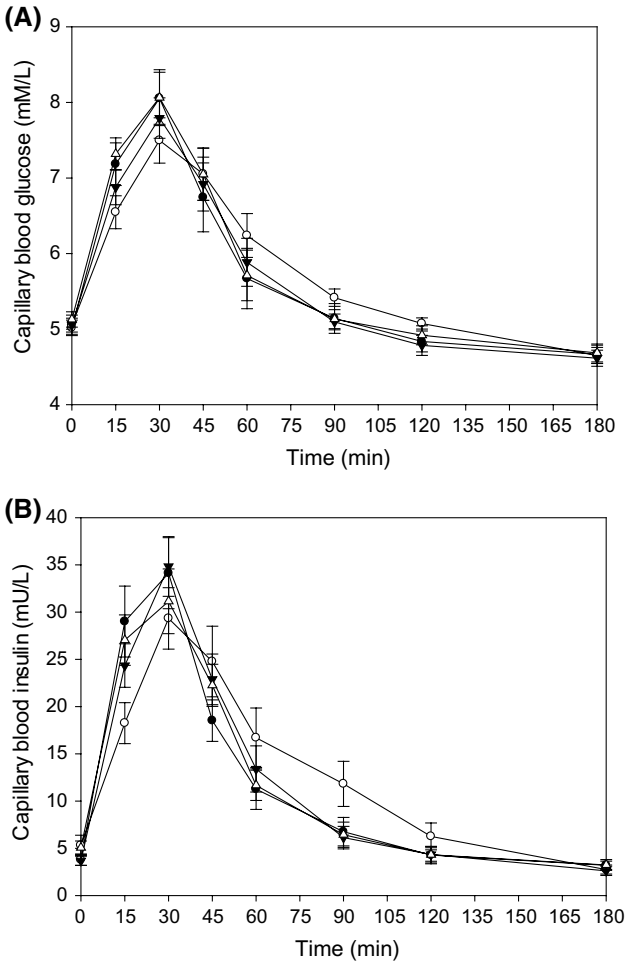

Fig. 2 Mean changes in plasma glucose (**a**) and insulin (**b**) in healthy subjects ($n = 13 \pm$ SEM) after the consumption of the study meals. Mashed potato (*filled circle*), mashed potato with broccoli (*open circle*), mashed potato with broccoli fibre (*upside-down filled triangle*) and mashed potato with cellulose (*open triangle*)

its nutritional characteristics when eaten is also of likely importance. A vegetable consumed with a carbohydrate staple ought not to contribute an additional high load of available carbohydrates.

The actual mechanisms responsible whereby vegetable, such as broccoli, may have an impact on carbohydrate staple GR/IR are open to debate. The solid bulk of the hydrated broccoli may have delayed gastric emptying, as could have its slightly higher energy load, which in turn delays the absorption of glucose into the blood. Broccoli is rich in polyphenols and phenolic acids [20]. Flavonol glycosides, hydroxycinnamic acids, caffeic acid and ferulic acid are abundant [21] and may have a potential to inhibit digestive enzymes [22], such as pancreatic α-amylase, and/or inhibit active glucose uptake [23]. Other mechanisms related to the physical state of the broccoli in the small intestine may also be important. In an in vitro digestion and ileostomy study of cooked pureed carrot, the gross tissue structure of intact cells remained unchanged [24]. However, the material had swelled and pectin in the cell wall was thought to have been solubilized and then released during stomach–small intestine digestion, which facilitated subsequent movement of water into cell wall voids [24, 25]. A subsequent reduction in mass transfer through reduced diffusion and digesta mixing and decreased free water would ultimately result in delayed glucose uptake. A similar active role was proposed for swollen insoluble undigesible plant cell walls in recent in vitro studies with kiwifruit [26] and in rat studies with broccoli fibre [7] and tossa jute fibre [27]. Any fibre solubilized during digestion is undoubtedly also important, and it may contribute to any of increased viscosity, reduced mixing, and masking of starch and/or amylase inhibition, to name just a few of the hypothesized mechanisms.

Substitution of broccoli with minimally processed broccoli fibre had only a very weak effect on GR. Since the NSP content and composition in broccoli and minimally processed broccoli fibre in the meal were identical, factors other than simply the NSP content and composition would seem responsible for differences in GR/IR response. Release and subsequent loss of pectins may have been greater for cooked broccoli than in the minimally processed broccoli fibre. The latter was also evenly mixed into the potato, as a dehydrated powder before re-heating and consumption, compared to the broccoli, which was re-heated separately and eaten together with the potato. Larger particles of intact vegetable cell matter from the broccoli meal may in some way impact GR and IR as suggested above via delayed gastric emptying. Another reason for the difference could have been the dose of broccoli fibre was not high enough (ca. 5 % w/w of the meal) or it did not fully rehydrate to the same state as the cooked broccoli.

At very best in our work, cellulose had a very weak effect, if any, on GR/IR. Previous studies in rats and pigs have shown that inclusion of solid particles, including crystalline cellulose, may reduce GR by increasing viscosity in the lumen [28, 29]. This effect is considered both particle size and dose dependent. Other in vitro studies, have recently shown insoluble dietary fibre additions, including wood fibre, to pre-gelatinized starch suspensions may act by sequestration or inhibition of amylase and that lumen viscosity may not fully explain how insoluble fibres might impact GR [30].

In a study with mashed potato mixed meals combined addition of rapeseed oil and salad attenuated, the strong increase in IR in healthy subjects induced by protein, from chicken breast, added alone [2]. A similar effect was observed when bok choy and oil where eaten together with chicken and rice [3]. However, in both these studies, fat and vegetable were added to protein and carbohydrate staple

together. It was, therefore, not possible to separate the individual effect on IR or GR of vegetable addition to a complex meal. Separate addition of spinach, but not carrots, Brussel sprouts or peas to a complex potato-based meal elicited a significantly lower IR in healthy subjects than a mixed meal without spinach [4]. The same was found for GR where spinach was added at a higher dose [5]. This may point to a role for specific vegetable types in suppressing GR and IR when eaten in combination with other meal ingredients such as moderate amounts of protein and fat in a mixed meal setting. It has also been shown that addition of vegetable side dishes to a meal with a moderate-fat content may also decrease the postprandial GR in healthy subjects without a disproportionate increase in IR [6].

Conclusion

The current work demonstrates that the addition of cooked broccoli to mashed potato has a significant effect on GR and IR in healthy subjects compared with potato eaten alone. Both peak, incremental peak blood glucose and the immediate glycemic response were significantly reduced as was the immediate demand for insulin. The mechanisms responsible for this effect and their individual significance are open to discussion. The results of our study suggest that addition of broccoli to potato improves glucose homeostasis and therefore indicates a physiologically significant role for vegetables when eaten together with a carbohydrate staple. The ratio of vegetable to carbohydrate staple is important, as is probably the type of vegetable and the way it is prepared prior to consumption. Since potato has a favourable content of nutrients over rice and pasta, its consumption as a carbohydrate staple corner stone of a mixed meal should be encouraged. Addition of a large portion of vegetables, such as broccoli, as part of a meal, which in turn is part of a balanced diet, is likely to confer both long- and short-term beneficial effects on health.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards All human studies have been approved by the appropriate ethics committee and therefore have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent All persons participating in the clinical study gave informed consent prior to their inclusion.

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