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ORIGINAL CONTRIBUTION

Evaluation of gastric processing and duodenal digestion of starch in six cereal meals on the associated glycaemic response using an adult fasted dynamic gastric model

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Abstract
Purpose To identify the key parameters involved in cereal starch digestion and associated glycaemic response by the utilisation of a dynamic gastro-duodenal digestion model.
Methods Potential plasma glucose loading curves for each meal were calculated and fitted to an exponential function. The area under the curve (AUC) from 0 to 120 min and total digestible starch was used to calculate an in vitro glycaemic index (GI) value normalised against white bread. Microscopy was additionally used to examine cereal samples collected in vitro at different stages of gastric and duodenal digestion.
Results Where in vivo GI data were available (4 out of 6 cereal meals) no significant difference was observed between these values and the corresponding calculated in vitro GI value.
Conclusion It is possible to simulate an in vivo glycaemic response for cereals when the gastric emptying rate (duodenal loading) and kinetics of digestible starch hydrolysis in the duodenum are known.

Keywords Starch · Glycaemic index · Barley · Oat

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Introduction

Cereal grains are the largest contributor to carbohydrate intake [1]. The only major source of glycaemic carbohydrate is digestible starch [2]. Both the rate of production and rate of absorption of glucose in the duodenum derived from starch, up to a maximum absorptive capacity of approximately 1 g/min [3], are largely dependent on the amount of starch per unit time entering the duodenum from the stomach [4], that is, gastric emptying, and the accessibility of that starch to pancreatic α-amylase [5]. The rate of gastric emptying is largely a function of the caloric density, nutrient profile and volume of the ingested meal [6]. While pancreatic α-amylase is in excess and not rate limiting [7], its accessibility to starch is a function of the physical structure and chemical composition of the food entering the duodenum [5]. The structure of food in the duodenum is, or may become, the rate limiting step in the production of amylolysis products (oligosaccharides > DP10). Since these oligosaccharides are water soluble and highly mobile they are rapidly hydrolysed into glucose by duodenal brush border membrane oligosaccharidases [7, 8]. Glucose is then rapidly and completely absorbed through the duodenum and transferred via the portal vein to the liver and then out into the systemic circulation system [9]. The initial rate (0–120 min) of glucose appearance in the systemic circulation system from fasting baseline is

therefore a function of absorption from the duodenum. It roughly corresponds to a linear increase to maximum followed by an exponential decay back to baseline [10, 11] although the rate of absorption is an individual variable depending upon age and gender.

This mechanism, referred to as duodenal glucose loading, induces a glycaemic response in the body in the form of an excursion from baseline of plasma glucose. Excursion is a general function of glucose absorption minus clearance, if glycogenesis, gluconeogenesis etc. are ignored. This excursion lasts the duration of loading of glucose from the duodenum plus the time for plasma clearance (T½ = 10–20 min) [12, 13], which although it is insulin dependent, is mainly constant within an individual. Peak plasma glucose concentration therefore occurs during peak loading at about 20–30 min after ingestion which is about the same time gastric emptying is complete for a breakfast cereal meal of low energy load, or just after, since the entire bolus will then be subjected to amylolysis in the duodenum.

With these observations in mind it should be theoretically possible to simulate the in vitro glycaemic response of a cereal-based meal and calculate an in vitro glycaemic index based on the kinetics of digestible starch hydrolysis in the duodenal lumen attenuated to account for delivery within a 120 min period. Because the concept of GI is based on a comparative system within an individual, then there is an automatic assumption that glucose clearance is linear with plasma glucose concentration. The only time this relationship becomes nonlinear is when glucose is lost via the kidneys. In fact, insulin response gives data sets that closely mimic those obtained by the measurement of plasma glucose [14]. There should therefore be no need (i.e. it is not necessary) to take into account, or correct for, all the proceeding individual physiological responses leading to intra- and inter-individual variation in a plasma glycaemic response as is the case for two recently proposed alternative in vitro digestion models [15, 16] that predict glycaemic response if one just wants to determine an in vitro GI. Some of these variable physiological responses include differences in rates of duodenal glucose absorption and plasma glucose disposal which in turn are individual variables based on insulin production, insulin sensitivity, body mass, age, muscle glycogen stores to name just a few.

To test the hypothesis that the kinetics of digestible starch hydrolysis in the duodenum attenuated to account for delivery over a 120 min period can predict the glycaemic response of cereals in vivo, we have utilised a newly developed dynamic gastric model [17] and a simple static model of the duodenum. We measure starch digestion as a function of digestion time. We examine six simple cereal meals. In vitro calculated theoretical glycaemic responses and index are compared to those measured for four of the same meals in vivo using the standard FAO/WHO protocol [18]. In order to gain new insights into the gastric processing of cereal grains, sub-samples of digesta were also collected upon simulated gastric empting and duodenal digestion and studied under the microscope.

Materials and methods

Grain processing

Olve barley and Belinda oat were processed on an industrial scale at Lantmännen Cerealia, Moss, Norway. For barley, the production process was as follows: sorting, cleaning, de-hulling and pearling to 75 % original mass. This was then micronized with infrared heating for 1 min at 95 °C and flaked to yield *micronized barley flakes* of 1.25 ± 0.13 mm thickness, 6.3 ± 0.83 mm width and 8.3 ± 0.83 mm length (all values mean of $n = 100$). Alternatively the pearled barley was subject to hammer milling to produce a flour which was moistened to 11.5 % (w/w) with water and extruded into flakes *extruded barley flakes* via a Bühler BI-EX Ø62 twin screw extruder (Extruder parameters: die dimensions, 9 × 3.5 mm; die temp, 105 °C; screw speed, 55 rpm; pressure, 40 bar; rate of flour addition, 200 kg/h). The product was immediately dried at 160 °C for 4 min to final moisture content of ca. 2.5 %.

Oat was processed as follows: sorting, cleaning, de-hulling, scouring and separating to yield groats (75 % of original mass). These were then treated in a kiln to inactivate fat-hydrolysis enzymes at 83 °C and 18–20 % moisture at the top and 21 °C and 10–12 % moisture at the bottom. Next the groats were heated, flaked and hammer milled to yield a whole grain *oat flour* (<1 mm particle size). The flour was then extruded and made into flakes (*extruded oat flakes*) in an identical manor to barley with a final moisture content of ca. 5.5 %. All samples were stored at 4 °C in a controlled humidity room in the dark prior to use.

Degree of starch gelatinization

This was determined by measuring the heat of starch gelatinization (transition enthalpy ΔH) in industrially processed samples by differential scanning calorimetry (DSC) [19] on a Mettler Toledo DSC823^e. Milled cereal samples (<0.5 mm) of ca. 30 mg were weighed into a 120 µl stainless steel pan followed by the addition of 60 µl of water. Milled pearled barley and oat groats from the same batch used to make the cereal samples were used as reference materials to normalise against ungelatinised starch. The DSC scanning rate was 5 °C/min from 10 to 120 °C.

An equivalent steel pan containing silicon oil was used as reference. The instrument was calibrated prior to use against indium. ΔH was calculated as the integrated area of the first peak in the thermogram per gram total starch (starch determined as described below and expressed in terms of dry weight) using STAR SW 9.01 software along with gelatinization onset temperature (T_o), peak temperature (T_p) and end set temperature (T_e).

Determination of starch, non-starch polysaccharide and beta-glucan

Starch was determined by AACC standard method 76.13 (without the use of DMSO or ethanol wash) using a Megazyme kit (Megazyme, Wicklow, Ireland). Non-starch polysaccharide (NSP) was determined by the method of Englyst [20] using CRM 514 breakfast bran as a positive control. Mixed-linkage beta-glucan was via a Megazyme kit according to AACC method 32.23.

Determination of moisture, protein and fat

Moisture content was determined on milled samples in a Sartorius Thermo Control YTC 01 L infra-red dryer. Protein was determined as total nitrogen × 6.25 in a Fison EA 1108 elemental analyser [21]. Fat was determined by accelerated solvent extraction [22].

In vitro dynamic gastric digestion of test meals

Test meals comprised 37.5 g cereal product mixed with 200 g water. This provided a meal of approximately 590 kJ (140 kcal) for the oat meal and 558 kJ (133 kcal) for the barley meal. These values are typical of the recommended breakfast cereal meal of around 35–40 g (approx. 135 kcal, 567 kJ) depending on the manufacturer. White bread (70 % extraction flour) corrected for water content and Kellogg's Cornflakes were used as control meals. Upon addition of water the cereal meals were left to stand for 2 min to ensure constant hydration. The bread was used as received. The meal was then eaten by one subject, but instead of swallowing the chewed boluses were spat out into a beaker. Once all the meal had been chewed, it was transferred to the stomach of the dynamic gastric model (DGM) already primed with 20 ml acidified salt solution (0.02 M HCl, 0.08 M NaCl, 0.03 M $CaCl_2$ and 0.9 mM NaH_2PO_4) for simulated gastric processing. The simulated conditions were as for a normal fasted adult with physiological and peripheral addition of acid and gastric enzymes, mixing, shear and residence time. Full details of the DGM that include its design, its function, the method to prepare simulated gastric enzyme secretions, in vitro—in vivo validation have been previously described [17, 23, 24]. The gastric process was started after a 1-min delay to allow the pH electrode in the 'main body' of the DGM model to equilibrate. Initial starting conditions in the model were constant. The total residence time of the meals was approximately 30 min during which samples of 30–40 ml of gastric digesta were ejected from the antrum every 5–6 min. Samples were denoted G (gastric) 1–6 D (duodenal) 0 indicating no duodenal processing.

Sample weight and pH were immediately recorded. The antral effluent was neutralised to pH 6.8 with saturated sodium hydrogen carbonate and re-weighed. A 5-g subsample was removed for simulated static duodenal digestion, 25 ml for determination of viscosity using a TA Instruments AR 2000 with a cup and modified bob (vaned) at shear rates between 0 and 500 s^{-1}, and 1 ml fixed in 3 ml of 0.1 M cacodylate buffer containing 3 % glutaraldehyde and stored at 4 °C for subsequent microscopic examination.

Static duodenal digestion

Duodenal digestion was conducted for 1 h at 37 °C following the physiological addition of simulated bile salts and pancreatic juice. The amounts added are calculated based on in vivo measured flow rates [25]. The recipe to make the simulated bile salts and pancreatic juice is described in full in [17]. Duplicate samples were removed from simulated duodenal incubation after 2, 5, 10, 15, 20, 30 and 60 min and denoted as a code of GxDy where x = the gastric digest sample identity (1–6) and y = the duodenal digest incubation time in minutes. These samples were either fixed for microscopy as described above or used to assay for in vitro digested starch as the 80 % ethanol soluble glucose oligosaccharides.

For the latter, the sample was brought to 80 % v/v with EtOH and left to stand at 4 °C overnight to precipitate the polysaccharide fraction. The samples were then centrifuged and 1 ml supernatant transferred to a clean dry screw top glass tube. This was dried under a stream of nitrogen at a maximum temperature of 50 °C. The dried sample was then treated with amyloglucosidase to convert the starch oligomers to glucose which was determined via the glucose oxidase–peroxidase assay (Megazyme, Wicklow, Ireland). The remaining supernatant was drained off and the precipitate analysed for total starch as described above.

Light microscopy and image analysis

The fixed digest suspension was vortex mixed for 10 s. Then 40–100 µl was pipetted out from the centre of the suspension and spread out onto a glass microscope slide. These were dried overnight in a fume cupboard. The slides were then mounted and examined under polarised light, in

epifluorescence mode for autofluorescence with blue light of 450–490 nm for excitation and a long band pass filter (>520 nm) used for visualisation of induced fluorescence [26], and after iodine-potassium iodide (IKI) staining [27]. All preparations were examined with a Leica DMR light microscope and typical images photographed with a Leica DC3000 CCD camera. Image resolution was 2,088 × 1,550 pixels with a scale of 2.895 pixels/μm.

The visual texture of images at a magnification of 10× obtained from IKI stained digests of micronized barley flakes and oat flour was assessed [28]. All images were processed and analysed by image analysis software (ImageJ, NIH-USA). The obtained digital images were divided into pixels (in our case 2,088 × 1,550 pixels) of which individual pixels take a value between 0 (black) and 255 (white) (8 bits grey level). Using the Isodata algorithm [28] the pixels in the monochrome images were allocated values such that the iodine stained starch was allocated black and the background white. The area fraction of IKI stained starch was calculated as a percentage of pixels in the image that have been allocated black.

Scanning electron microscopy (SEM)

The fixed digest suspension was vortex mixed for 10 s. Then 300 μl was pipetted out from the centre of the suspension and transferred to a plastic tube covered at each end with microscope lens paper. This was submerged under 0.05 M PIPES buffer, pH 7.2 followed by a graded series of alcohols for sample dehydration. Samples were then dried in a critical-point dryer (BAL-TEC CPD 030) under CO_2, mounted on aluminium stubs and finally sputter coated (Polaron SC 7640) twice with gold–palladium. The samples were viewed in a Zeiss EVO-50-EP SEM.

Calculation of in vitro potential plasma glucose loading curves and GI values

All in vitro GI data were gathered blind to the in vivo results and are based on the rate of production of low molecular weight starch products (<DP10) by the pancreatic α-amylase in the presence of trypsin, chymotrypsin and lipase. It is assumed that there is no impediment to this fraction being further depolymerised in the lumen or at the brush border, and there is no impediment to absorption of these mobile water soluble components. This assumption is based on very similar time base of plasma glucose curves obtained with cooked starches and glucose [29, 30]. The in vitro data therefore represent the potential plasma loading curves (rate of creation of bioaccessible starch hydrolysis products), whereas GI measured in vivo is a combination of the plasma loading and clearance curves, clearance being an individual variable.

The rate of production of oligomers (mass per unit time) in each gastric sample was plotted and a straight line fitted to the early linear part of the curve to obtain a maximum rate for each sample. Duration of the production of the starch oligomers in each sample was obtained by dividing the total weight of starch by the rate of hydrolysis (as measured by the rate of creation of starch oligomers). The total rate of production (mg min^{-1}) as a function of time for the whole meal was found by the addition of the individual rate (mg min^{-1}) data as in the shortened example shown in Table 1. The total rate plot (y) against time (x) was fitted with the exponential function $y = x^{a}e^{-bx}$ using the Marquardt–Levenberg algorithm [31] in SigmaPlot 11 (Systat Software Inc). The area under the curve (AUC) from 0 to 120 min was found by integration using the trapezoidal method. The AUC was then normalised against the total in vitro experimentally determined digestible starch content of white bread for comparison of equal starch loads. The 120 min AUC for white bread was allocated a GI of 100. GI of food = (120 min AUC food normalised for in vitro digestible starch content against white bread/120 min AUC white bread) × 100.

In vivo GI measurements of barley test meals

These measurements were taken by Leatherhead Food Research, Leatherhead, Surrey, UK, and followed the FAO/WHO protocol [18]. Twelve healthy subjects between 18 and 65 years of age took part in the study. Informed consent was obtained from each individual. Each subject had a similar meal prior to each morning test session. The test meal and glucose reference meal were tested in each subject in duplicate and triplicate, respectively. The study was randomised to remove any order effects. Each subject received one portion of a test meal containing 25 g available carbohydrate in which the whole amount was consumed in a 15 min period. This smaller amount of available carbohydrate, rather than the standard 50 g, was necessary for each meal so they would be manageable to consume without milk. The two meals comprised 46 g micronized barley flakes or 41 g extruded barley flakes in 200 ml water. The glucose reference meal consisted of 25 g glucose dissolved in 250 ml water. After starting the consumption of the meal ($T = 0$) blood samples were taken at 15 min intervals for the first hour and then at 30 min intervals up to 2 h. Blood was collected into small tubes containing lithium–heparin following a finger-prick. Tubes were immediately mixed and centrifuged at 3,000 rpm for 10 min to separate the plasma. Blood glucose was then measured via a YSI 2300 Stat Plus Glucose and Lactate Analyser. Data analysis was analysed by the standard WHO/FAO protocol [18]. The GI calculated from the glucose reference (±SEM) was converted to a white

Table 1 A shortened example of the computation of the total rate of production of starch oligomers (mg min^{-1}) as a function of time from the whole cereal meal through summation of the individual rate data

Time (min)	Gastric 1 rate A	Gastric 2 rate B	Gastric 3 rate C	Gastric 4 rate D	Gastric 5 rate E	Gastric 6 rate F	Total rate Σ
6	A						A
	A						A
11	A	B					AB
	A	B					AB
16	A	B	C				ABC
	A	B	C				ABC
21	A	B	C	D			ABCD
	A	B	C	D			ABCD
26		B	C	D	E		BCDE
			C	D	E		CDE
31				D	E	F	DEF
				D	E	F	DEF
					E	F	EF
					E	F	EF
						F	F
						F	F

bread reference GI by dividing the glucose reference result by 0.71 [32].

Statistical comparison of GI values

A one sample *t* test was used to compare in vivo GI values with an estimated in vitro GI value taken from the analysis of one such sample, unfortunately without any information about variance or measurement uncertainty for the latter.

Results

Characteristics of cereal products

For the cereal products in this study food structure and composition is typical in terms of their industrial processing and state upon consumption (Table 2). When differences in moisture content are accounted for, the starch content in extruded and non-extruded processed oat and barley flakes is similar (Table 2). Extrusion resulted in moisture loss, with a corresponding increase in fresh-weight starch content, and starch gelatinisation to a degree of 82 and 93 %, respectively (Table 3). Neither displayed birefringence in the form of a Maltese-cross under cross-polarised light (result not shown). In contrast very little gelatinization (11 %) occurred during the production of oat flour, while micronisation of barley with infrared heating at 95 °C for 1 min resulted in gelatinisation of just over a quarter of starch granules by comparison with pearled barley (Table 3). Birefringence was evident in both while gelled starch was not definitively observed under the light microscope. Cornflakes and white bread represent typical starch-rich meals, very low in dietary fibre and fat, with a lower protein content (Table 2) on an 'as consumed' basis.

The contents of non-starch polysaccharide were about 9/100 g for processed oat and 13/100 g for processed barley (Table 2). β-glucan content, as part of NSP, was almost identical for both processed grains at 4.9–5.6/100 g. Extrusion had no effect on total NSP or β-glucan content. Oat products contained slightly more protein than the barley products but all where between 10.5 and 14.8/100 g. As expected oat products contained more fat, 6–7/100 g, than the barley products which contained 0.5–1.8 g/100 g (Table 2). All foods could be readily broken down by chewing although micronised barley flakes were the most resistant.

Viscosity of antral digesta and antral pH

The viscosity of the digesta ejected from the antrum (entering the duodenum) was measured between 1 and 500 s^{-1}. The viscosity at a nominal antral shear rate of 26 s^{-1} was taken to be the viscosity experienced in the antrum. For all cereal meals and gastric emptying times, the viscosity was generally low and less than 2 Pa s. The viscosity of antral digesta from the micronised barley flake meal decreased from over 2 to <0.01 Pa s during the course of digestion (Fig. 1a). The initial antral digesta viscosity of the oat flour meal was an order of magnitude smaller and by the latter stages of gastric digestion it was similar to the barley flake meal (Fig. 1a). Antral digesta derived from the extruded barley flake meal had a slightly greater viscosity than the oat flour in the early stages of

Table 2 Main nutritional composition of cereal products (% fresh weight)

	White bread[a]	Cornflakes[a]	Micronised barley flakes	Extruded barley	Oat flour	Extruded oat
Moisture	40.4	3.0	10.8	2.8	10.7	5.5
Protein	7.6	7.9	10.5	11.4	14.8	13.5
Fat	1.3	0.7	1.8	0.5	6.1	6.7
Starch	46.7	77.7	54.3	60.2	49.7	55.4
NSP (of which is β-glucan)	1.5	0.9	12.6 (5.1)	14.5 (5.6)	9.4 (4.9)	8.9 (5.4)
Σ	97.5	90.2	90	89.4	93.3	95.2

[a] Values from [2]

Table 3 Mean ($n = 2$), onset (T_o), peak (T_p), conclusion (T_e) temperature, enthalpy of gelatinisation (ΔH) and degree of gelatinization of industrially processed oat and barley products

	T_o (°C)		T_p (°C)		T_c (°C)		ΔH (J/g starch)		Gelatinisation (%)
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
Oat groats	56.08	0.07	63.89	0.06	74.31	0.11	6.39	0.20	0
Flour	58.86	0.007	66.03	0.03	75.88	0.02	5.7	0.08	11
Extruded flakes	51.63	1.95	62.92	0.6	79.64	0.37	0.47	0.37	93
Pearled barley	58.59	0.212	67.03	0.21	80.63	0.39	6.07	0.16	0
Micronised flakes	57.23	0.18	66.62	0.15	79.12	0.37	4.42	0.04	27
Extruded flakes	49.27	1.56	63.92	2.52	83.77	0.66	1.1	0.04	82

digestion. The digesta viscosity then slightly increased before steadily decreasing, but to a level slightly higher than the oat and barley samples not subjected to extrusion (Fig. 1a). The viscosity of the antral digesta derived from the extruded barley flake meal showed a similar trend to that of extruded oat meal although the viscosities were roughly double (Fig. 1a). Similarly in the later stages of digestion the antral digesta viscosity of extruded barley was slightly greater (ca. 0.3 Pa s) than those of the micronised barley flake meal at corresponding times of gastric emptying (Fig. 1a). The viscosity of the first antral digesta from the white bread meal was 0.1 Pa s which was lower than the corresponding oat and barley antral samples. In the next two subsequent samples the viscosity increased to just under 0.5 Pa s and in the last two samples decreased to just less then that of the first gastric digesta. The antral viscosity of the cornflake meals was more constant throughout digestion between 0.11 and 0.23 Pa s.

In terms of antral pH this varied from 3.9 to 4.5 in the first 16 min of gastric digestion for the micronised barley flake meal before progressively dropping to 1.8 towards the end of gastric processing (Fig. 1b). A similar trend in antral pH was observed for the other oat, barley and white bread meals with an initial higher pH up to a maximum of 5.5–6 followed by an eventual decline in pH to around 2 as gastric digestion approached completion (Fig. 1b). It seemed cornflakes had a much lower initial pH buffering capacity in the antrum compared to other samples with pH only reaching a maximum of about 3.6 after 16 min processing before dropping back to about pH 2 towards the end of gastric processing (Fig. 1b).

Microscopy examination of antral digesta and duodenal digestion products

Antral digesta examined by light microscopy from all barley and oat meals contained various sizes (ca. 0.01–1 mm^2) of bran fragments. These have a blue/turquoise autofluorescence as typified by bits of aleurone (Fig. 2a, b). Oat samples contained smaller bran fragments than the barley samples although there appeared to be no major differences between extruded and non-extruded samples or as a function of digestion time.

Light microscopy examination also revealed an abundance of small and large round and elliptical barley starch granules in the micronised barley flake meal entering the gastric compartment and in the first gastric digesta collected between 1 and 16 min (Fig. 2c, d). They gradually decrease in numbers (Fig. 3a) as a function of increasing gastric digestion time. Most of these starch granules are absent after 27–33 min in the gastric compartment (Fig. 2e, f). While intact starch granules dominate in the first gastric sample with only a 6 min gastric digestion time (Fig. 4a), eroded and pitted granules increase in abundance in subsequent gastric samples (Fig. 4b, c) until only the bran fragments dominate in the material subject to the

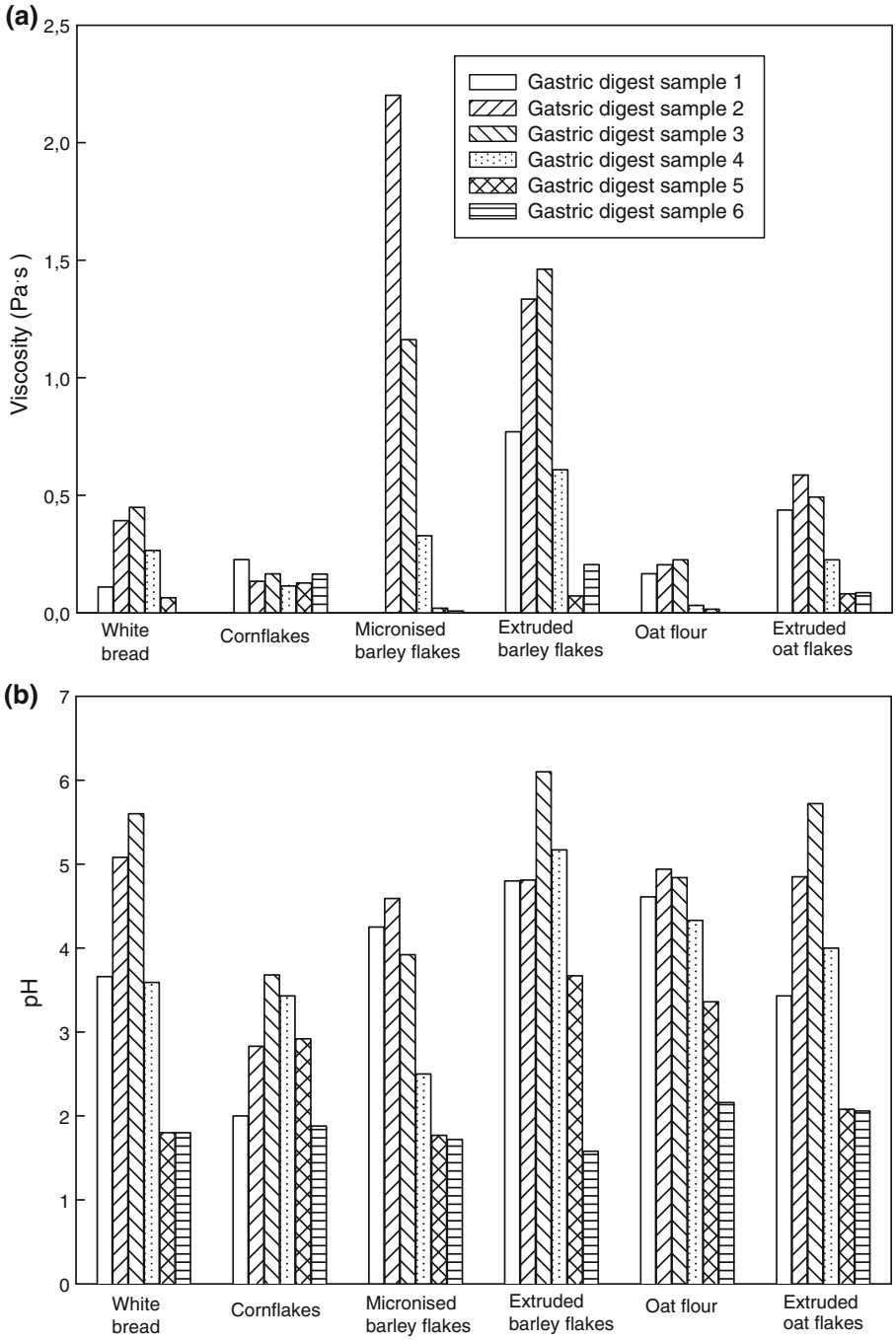

Fig. 1 Antral digesta apparent viscosity (**a**) at an antral shear rate of 26 s^{-1} and antral pH (**b**)

longest gastric digestion time (Fig. 4d). Changes in the abundance and appearance of small and large barley starch granules as a function of digestion time in the duodenum are much less pronounced (Figs. 2g, h, 3a, 4e, f) and they look similar to the gastric sample they originated from.

In the simulated digestion of the oat flour meal most oat starch granules occur as single granules or in clusters (Fig. 2i, j). In contrast to the micronised barley flake meal there is an abundance of starch granules in all the gastric digesta emptied from the stomach (Figs. 2j, 3b). However, it seems that the starch granules in the part of the meal that underwent the longest gastric digestion are more susceptible to further digestion in the duodenum (Figs. 2k, 4b) than starch granules that pass out of the gastric compartment at a much earlier stage (Figs. 2l, 4b). Oat starch granule clusters become less frequent as a function of digestion time.

The starch present in the extruded barley and oat is visualised in the first gastric emptying as dense blue structure-less clumps (Fig. 2m, n). The clumps become less

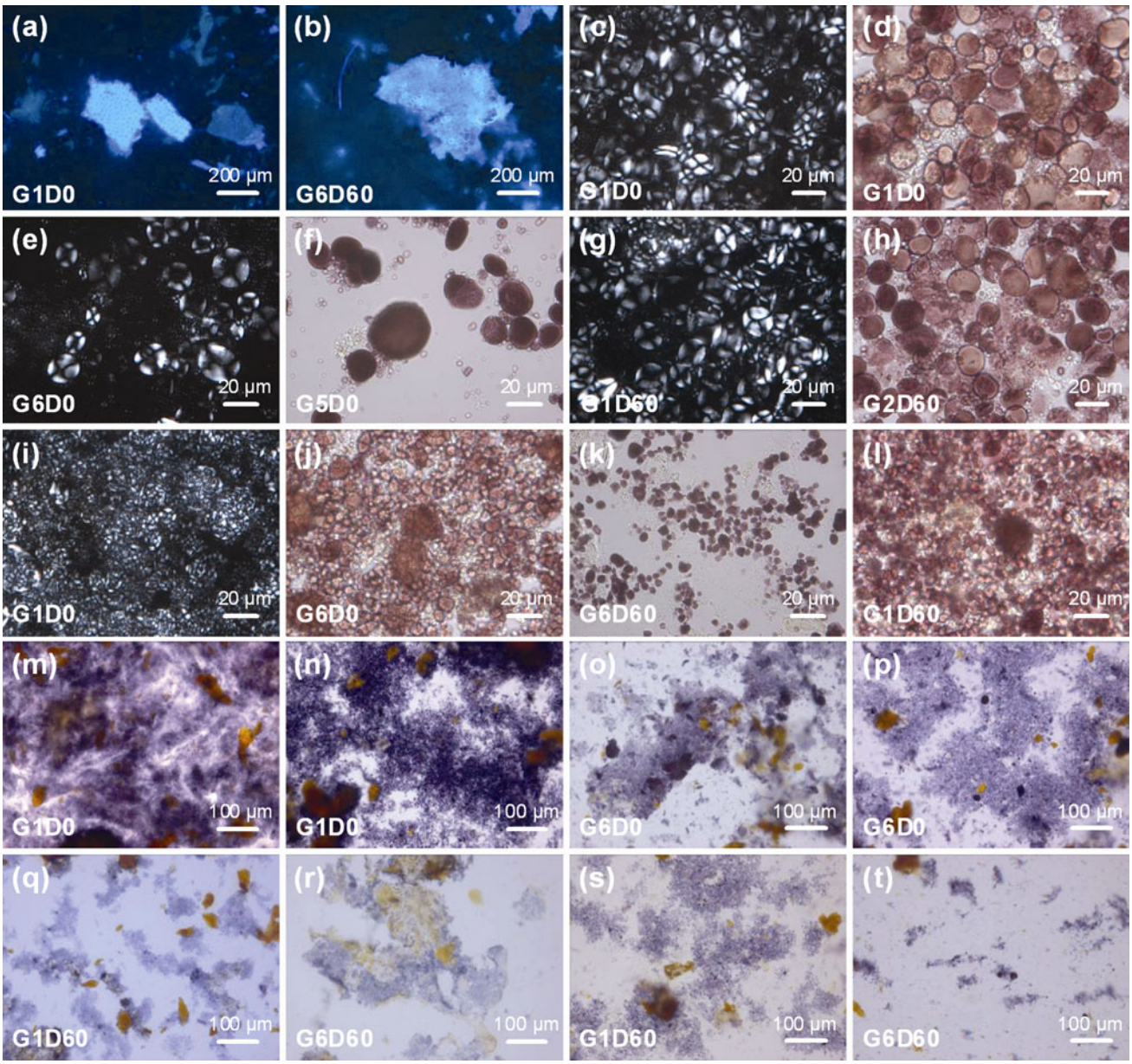

Fig. 2 Light micrographs of in vitro digests of Olve barley and Belinda oat meals examined for their autofluorescence (**a**, **b**), under polarised light (**c**, **e**, **g**, **i**) and after staining with potassium iodide-iodine (**d**, **f**, **h**, **j**–**t**). Micrographs **c**–**h** micronized barley flakes, **i**–**l** oat flour, **a**, **m**, **o**, **q**, **r** extruded barley flakes, **b**, **n**, **p**, **s**, **t** extruded oat flakes. The code in the *bottom left* hand corner of each micrograph denotes digestion status upon sampling (see 'Methods')

dense and more disperse as gastric digestion proceeds (Fig. 2o, p) for both meal types. Further starch digestion occurs in the duodenum (Fig. 2q–t) but it does not go to completion and some starch remains.

Glycaemic response and index

When white bread was used as the reference meal there was no significant difference ($p < 0.05$) between the calculated in vitro GI values and corresponding in vivo GI values (based on 120 min AUC and equivalent available carbohydrate content) for cornflakes and the two barley products (Table 4). Compared with the other test meals studied in vitro the micronised barley flake meal had a low digestible starch content, peak rate of starch hydrolysis and a long duration of starch digestion (Table 4; Fig. 5b). This resulted in an extended and flattened potential plasma glycaemic loading curve (Fig. 5b). The in vitro calculated GI was 66 and quite similar to that calculated in vivo (Table 4). The peak of the GI curve in plasma in vivo (Fig. 6) occurs around 30 min. Even though the fitted peak rate of starch hydrolysis occurs much later at 72 min

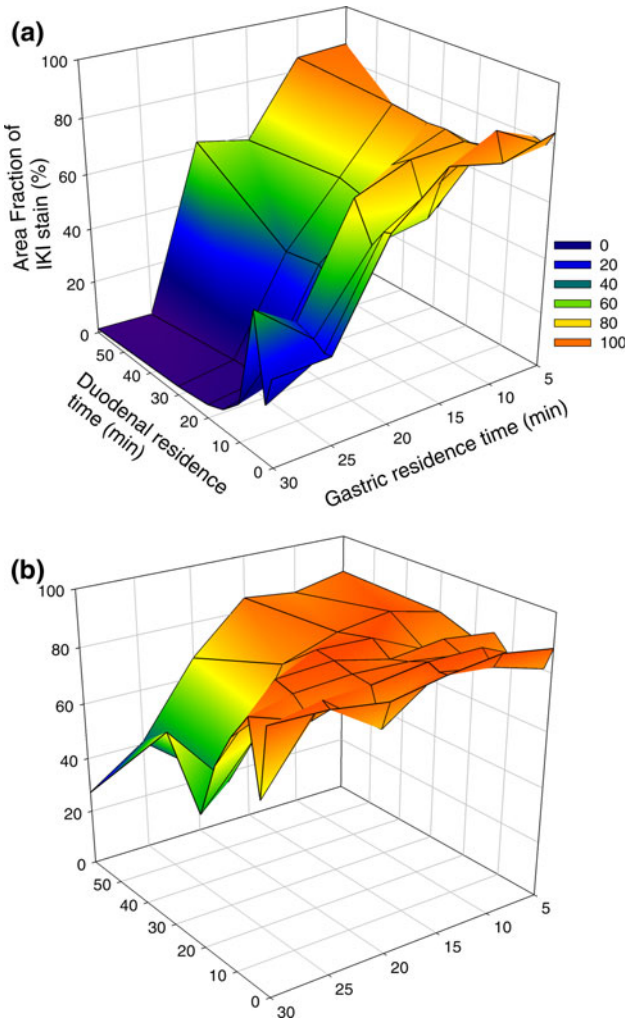

Fig. 3 Area fraction of image with IKI staining as a function of gastric and duodenal digestion time. **a** Micronized barley flakes and **b** oat flour

(Table 4), the rate is similar to that at 30 min (Fig. 6). The decline in plasma glucose seen in the glycaemic response curve in vivo occurs gradually from 30 min to beyond 150 min which is similar to that in vitro.

At the other extreme the cornflake and white bread meals had over double the content of digestible starch than the micronised barley flake meal and much greater peak starch hydrolysis rates of 462 and 346 mg min^{-1}, respectively (Table 4). This occurred within in a much shorter hydrolysis time of 112 and 154 min, respectively. Consequently the in vitro potential plasma glycaemic loading curves are sharp and narrow (Fig. 5) and the in vitro GI similarly high at 111 for cornflakes (Table 4). The plasma glucose curves for these two meals examined from the literature [14, 33] closely resemble the in vitro potential plasma glycaemic loading curves (Fig. 5b). As for the micronised barley flake meal peak plasma glucose occurs at 30 min while the fitted rate occurred at 47 min. For the cornflake meal the plasma glucose then rapidly declines to baseline by 90 min while for the white bread meal the decline is more gradual and protracted with a return to baseline >120 min [14, 33]. A similar trend is observed in vitro (Fig. 5).

For the oat flour meal the total digestible starch content was less than the white bread and cornflakes meals. But even though the duration of in vitro starch hydrolysis was similar to the micronised barley flake meal, the GI was much higher (Fig. 5c; Table 4). These higher values reflect a quite rapid rate of starch hydrolysis in the meal as well as the greater availability of total digestible starch. In contrast the extruded barley and oat flake meals contain almost half the total digestible starch content than the oat flour meal. But because this starch is rapidly digested (Fig. 5) the in vitro GI values are high at 103 and 105, respectively. It is not a surprise that the in vitro potential plasma glycaemic loading curves look similar to each other (Fig. 5a) and are slightly right skewed (Fig. 5). This pattern is reflected in the plasma glucose curve for the extruded barley meal (Fig. 6) where there is a shorter but greater excursion of plasma glucose ending at about 90–100 min. The maximum rate of loading in vitro for the extruded barley meal also coincides with the plasma glucose peak maximum in the in vivo GI curve (Fig. 6).

Discussion

Our study shows it is possible to simulate the in vivo glycaemic response of a simple starch-rich cereal meal in vitro when only the kinetics of digestible starch hydrolysis in the duodenum are known and these are attenuated to account for delivery from the stomach. It has recently been shown that in vitro starch digestion kinetics can accurately predict portal glucose appearance as a glycaemic response up to 8 h postprandial when gastric emptying is accounted for [34]. The relatively small energy loading of the cereal-based breakfast meals in our study results in a relatively short total gastric residence time. It is nevertheless essential that the duration of gastric processing time corresponds to the typical in vivo state so as to accurately simulate the starch digestion kinetics in the duodenum from the starting point of meal ingestion. In this way one can attempt to simulate a postprandial glycaemic response over 120 min expressed in terms of potential plasma glucose loading.

Our results also show that it is sufficient only one individual chews the meal and expectorates it just prior to swallowing. This is because there is no significant inter-individual variability in the particle size of food boluses at the end of chewing [35] and the contribution of salivary α-amylase to plasma glucose loading is rapidly nullified by the action of pancreatic α-amylase in the duodenum [36].

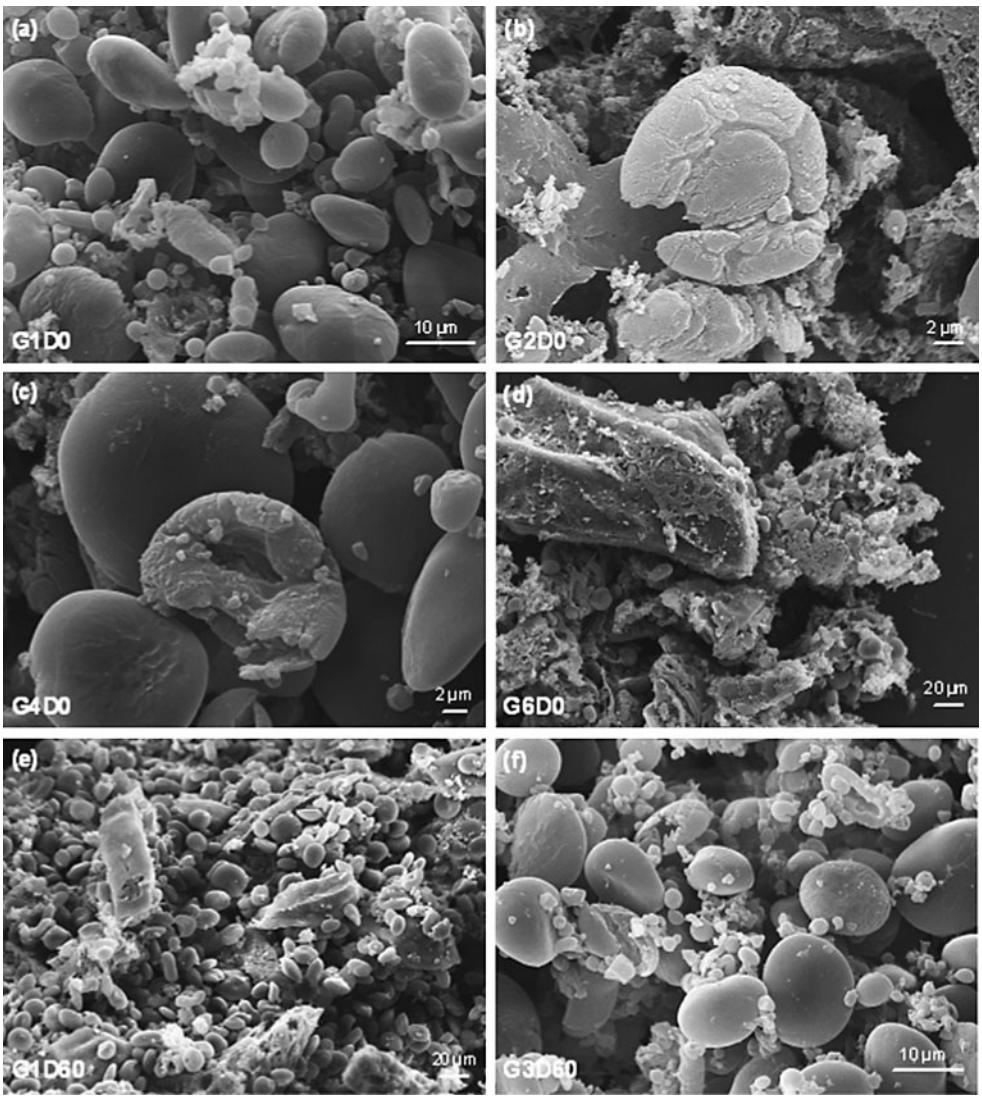

Fig. 4 Scanning electron micrographs of in vitro digests of micronized barley flakes (**a**–**f**). The code in the *bottom left*-hand corner of each micrograph denotes digestion status upon sampling (see 'Methods')

Table 4 In vitro modelled characteristics of starch hydrolysis for six cereal meals

Food	Peak rate (mg min^{-1})	Peak time (min)	Total digestible starch (g)	In vitro GI	In vivo GI[a]	
					Mean	SEM
White bread	346	54	28.1	100	100	
Cornflakes	462	47	28.7	118	116[b]	5[b]
Oat flour	221	59	20.5	92	N.D.	
Extruded barley flakes	205	37	12.6	103	96.6	7[c]
Micronised barley flakes	92	72	12.8	66	70	6[c]
Extruded oat flakes	204	39	11.1	105	N.D.	

ND not done

[a] According to FAO/WHO protocol [18]

[b] Value from an international table of glycaemic index. Mean SEM of 5 studies [45]

[c] SEM of 1 study $n = 12$

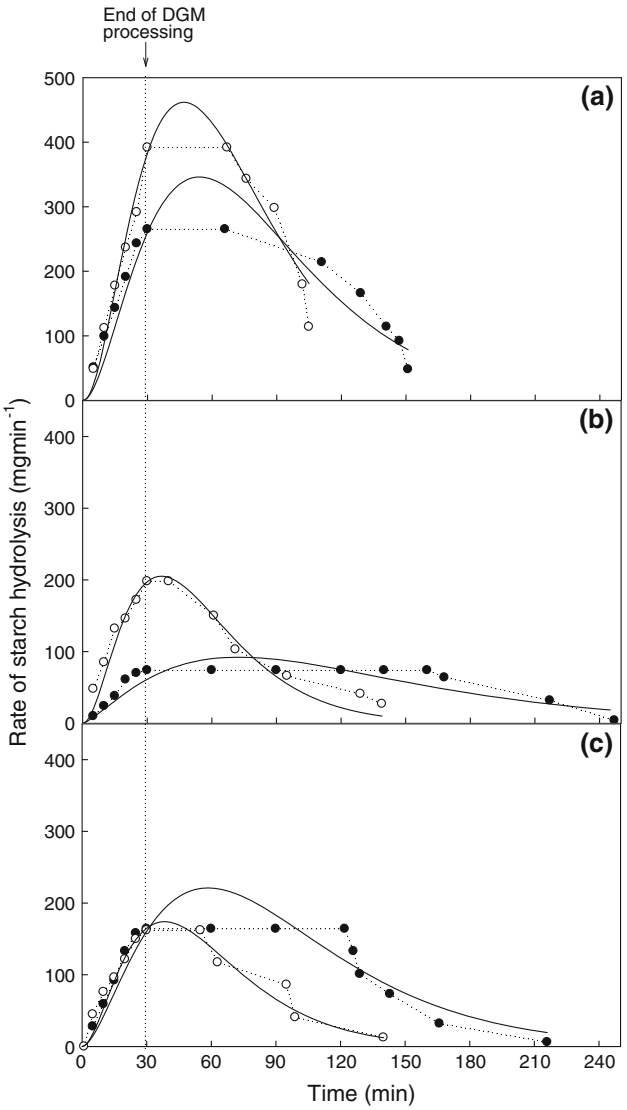

Fig. 5 Potential glycaemic plasma loading curves expressed as the rate of starch hydrolysis. White bread and cornflakes (**a**), micronised barley flakes and extruded barley flakes (**b**), oat flour and extruded oat flakes (**c**). *Solid and open circles,* respectively. *Solid lines* are the curve fits

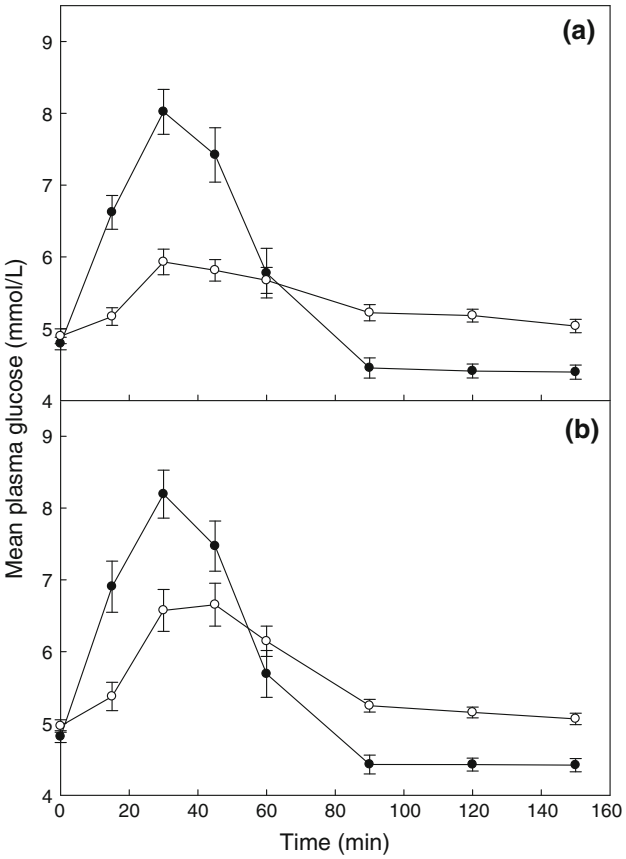

Fig. 6 Mean (±SEM) plasma glucose determined for in vivo GI measurements for micronised barley flakes (**a**) and extruded barley flakes (**b**). *Solid circles* are the glucose reference

Furthermore, when in vivo GI is determined by a panel of volunteers it is independent of the person chewing since: (1) it is a comparison within an individual and (2) it is a mean across a number of panellists. We demonstrate that the DGM and associated duodenal model provide data sets that can be used to describe the GI of some cereals. Most importantly, GI is a comparative indexation method where there is a standard (100 %) against which all others are judged. Each subject in a volunteer panel therefore provides a data set for the standard and the test material, and the two AUC's for plasma glucose are compared within an individual (mastication and gastric emptying differences are nullified). Relative GI's are then averaged across the panel. The fact that only one person chewed the food is not different to the contribution of a single individual to a panel where the mean data are derived.

Recently two further in vitro digestion models of glycaemic response have been proposed [15, 16]. They both attempt to take into account the complex and highly individually variable physiological processes occurring outside of the duodenal lumen postdigestion. 'TIM-Carbo' applies a homeostatic model to predict the in vivo glycaemic response in blood plasma after carbohydrate ingestion [16] while the 'glycaemic impact' model attempts to take into account the homeostatic response of blood glucose clearance [15]. Our approach, however, avoids all the physiological factors that results in a high inter- and intra-individual variation in glycaemic response.

A whole host of other in vitro carbohydrate digestion models exists that can predict the glycaemic properties of foods [37] but they do not simulate the glycaemic response over time and seldom accurately replicate the biological digestive processes in the human especially with respect to dynamic gastric processing. They often only predict a GI value based on chemically operational definitions [37, 38]

such as in the concept of rapidly and slowly digestible starch [1, 39]. In order to predict GI one really needs to take into account the rate of gastric emptying in addition to the total load of available carbohydrate. Apart from at breakfast, carbohydrates are usually consumed as part of a complex meal. In the postprandial state where the other components of the meal, particularly fat [40], have an impact on the rate of gastric emptying the standard concept of GI becomes meaningless. The glycaemic response under such circumstances is overwhelmingly dictated by the rate of gastric emptying rather than any changes in the accessibility or digestibility of starch. As a consequence it is possible to convert a high GI food into a low GI food, not by changing the inherent digestibility of the starch in the duodenum, but simply by reducing the rate of delivery from the stomach.

It is well-known heat treatment of cereals during processing and product preparation greatly increases the accessibility of starch to hydrolysis by pancreatic α-amylase. Even minimal processing such as flaking (steaming and rolling) of cereal kernels can greatly increase the rate of production of starch degradation products during digestion compared to whole cereal grains [41]. This is despite only a fraction of the starch is gelatinised and most of the botanical structure is intact. On the other hand milling grains to produce flour mechanically disrupts most cell structure. As a consequence the starch is more readily accessible and susceptible to α-amylase degradation during digestion than flakes. Similarly the starch in extruded flours, which undergoes both heat treatment and mechanical disruption, is also readily susceptible to attack by α-amylase.

It is therefore perhaps not a surprise that all the cereal meals in this study, with the exception of the micronised barley flake meal, induced plasma glycaemic responses similar to, or only slightly less than, white bread. Although the oat meals where not assessed for their in vivo GI properties, other studies on extruded cereals and flours show they are high and similar to white bread [41]. In common with flaked products in general, the micronized barley flakes in this study, still induce an overall high glycaemic response (GI $>$ 70).

For the micronised barley flake meal, and although it is insufficient to be discriminated from baseline, we speculate that beyond 150 min there is a continuous bleeding of slowly digestible glucose. Such observations from in vitro modelling of the slower rate, and longer duration, of small intestinal starch hydrolysis in our study support this hypothesis. In a study of the kinetics of glucose appearance and disposal in the blood following a starch-rich meal of cooked peas a significant appearance of glucose post 120 min was derived from slowly digestible starch [10]. This extra glucose loading would be 'invisible' on procedures only relying on measuring blood glucose concentrations due to the homeostatic action of insulin and the concomitant drop in endogenous glucose production [10]. Hence, the excursion of blood glucose from baseline in the standard concept of GI is restricted to 120-min post-ingestion after a prolonged period of fasting.

Whereas the bolus formed upon chewing and swallowing of the other processed and readily hydrated cereal meals is a soft and a easily dispersed slurry of small particles (<2 mm), the bolus formed upon swallowing the micronised barley flake meal was more heterogeneous and also comprised large botanically intact cereal pieces. This has been noted earlier in barley flake meal samples collected from ileostomy subjects [42]. In the antrum the swallowed food is subjected to an element of selective sieving. Accordingly the largest particles (>2 mm) tend to be retained until the latter phase of gastric emptying. Larger pieces remaining in the antrum after gastric processing are eventually emptied by the 'housekeeper' migrating motor complex, type III wave during fasting. However sieving does not explain the reduction in starch granules in the digesta of the micronised barley flake meal collected from the antrum as a function of gastric emptying.

Additionally the size and density of particles and the viscosity of the matrix in the low mixing environment of the main body of the stomach may also contribute to the population profile of particles which are emptied over time such that small dense starch granules are emptied first. While cold swelling of dried cooked starch is typical for extruded and thermally processed cereals and was observed in this study as an increase in initial antral viscosity, it was not very pronounced for the micronised barley flake meal whose antral viscosity profile was dominated by the initial status of the bolus, its subsequent breakdown, and emptying from the antrum. For solid foods it is thought that meal viscosity has little influence on gastric emptying because of rapid intragastric dilution [43]. However, viscosity in the duodenum may be an important factor influencing the accessibility of pancreatic α-amylase to its starch substrate and hence an important factor in modulating glucose loading [44]. In the case of the cereals analysed in our study in vivo viscosity in the antrum was similar so viscosity in the duodenum was also probably similar and therefore not a factor explaining differences in starch digestibility.

Although some salivary α-amylase retains activity in the stomach it is thought to be somewhat diminished because α-amylase exposed to gastric secretions is rapidly degraded. Hence it is traditionally thought that salivary α-amylase plays a minor role in gastric starch hydrolysis [7] yet this still may be enough to account for the pits observed on some starch granules in digesta with the longest gastric residence time. Other buccal enzymes, such as lingual lipase, that are inactivated by low pH can retain their

activity for up to 1 h in the centre of the bolus because gastric contents are heterogeneous and often poorly mixed and therefore shielded from contact with the gastric secretions [44]. Perhaps in a similar manner α-amylase also remained active in parts of the micronised barley flake meal. At present this explanation is still somewhat speculative.

To ultimately confirm or refute some of the possible interpretations of the results from this study, it is quite clear more replicate experiments with different and more complex meals, for example, adding milk to cereals, are required. The results and approach used here also support and reinforce extensive earlier validation of the DGM against the typical in vivo state. Furthermore, and assuming the in vivo state is represented accurately, one can start to begin to understand the key processes that are involved in an associated biological response to an ingested food or meal. Some of these biological processes in relation to starch digestion in some simple cereal meals have been investigated and discussed in this study and present some new avenues for future research into understanding the role of starch digestion in health and disease. As a method of determining GI of foods it is currently too premature to propose our model as a viable alternative to standard in vivo GI testing. What is proposed here is an approach that needs further investigation and refinement.

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Conflict of interest The authors declare no conflict of interest.

References

1. Englyst KN, Vinoy S, Englyst HN, Lang V (2003) Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose. Br J Nutr 89(3):329–339. doi:10.1079/bjn2002786
2. McCance RA, Widdowson EM, Holland B (1991) McCance and Widdowson's the composition of foods, 5th edn. Royal Society of Chemistry, Cambridge
3. Jeukendrup AE (2004) Carbohydrate intake during exercise and performance. Nutrition 20(7–8):669–677. doi:10.1016/j.nut.2004.04.017
4. Mourot J, Thouvenot P, Couet C, Antoine J, Krobicka A, Debry G (1988) Relationship between the rate of gastric emptying and glucose and insulin responses to starchy foods in young healthy adults. Am J Clin Nutr 48(4):1035
5. Englyst KN, Englyst HN (2005) Carbohydrate bioavailability. Br J Nutr 94(1):1–11. doi:10.1079/bjn20051457
6. Calbet JAL, MacLean DA (1997) Role of caloric content on gastric emptying in humans. J Physiol (Lond) 498(2):553–559
7. Gray GM (1992) Starch digestion and absorption in nonruminants. J Nutr 122(1):172–177
8. Wahlqvist ML, Wilmshurst EG, Murton CR, Richardson EN (1978) Effect of chain-length on glucose-absorption and related metabolic response. Am J Clin Nutr 31(11):1998–2001
9. Livesey G, Wilson PDG, Roe MA, Faulks RM, Oram LM, Brown JC, Eagles J, Greenwood RH, Kennedy H (1998) Splanchnic retention of intraduodenal and intrajejunal glucose in healthy adults. Am J Physiol Endocrinol Metabol 275(4):E709–E716
10. Robertson MD, Livesey G, Mathers JC (2002) Quantitative kinetics of glucose appearance and disposal following a C-13-labelled starch-rich meal: comparison of male and female subjects. Br J Nutr 87(6):569–577. doi:10.1079/bjn2002586
11. 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(05):853–864
12. Amatuzio DS, Stutzman FL, Vanderbilt MJ, Nesbitt S (1953) Interpretation of the rapid intravenous glucose tolerance test in normal individuals and mild diabetes mellitus. J Clin Invest 32(5):428–435
13. Sjöstrand F, Hahn RG (2003) Validation of volume kinetic analysis of glucose 2.5 % solution given by intravenous infusion. Br J Anaesth 90(5):600–607. doi:10.1093/bja/aeg102
14. Lee BM, Wolever TMS (1998) Effect of glucose, sucrose and fructose on plasma glucose and insulin responses in normal humans: comparison with white bread. Eur J Clin Nutr 52(12):924–928
15. Monro JA, Mishra S (2010) Glycemic impact as a property of foods is accurately measured by an available carbohydrate method that mimics the glycemic response. J Nutr 140(7):1328–1334. doi:10.3945/jn.110.121210
16. Bellmann S, Minekus M, Zeijdner E, Verwei M, Sanders P, Basten W, Havenaar R (2010) TIM-Carbo: a rapid, cost-efficient and reliable in vitro method for glycemic response after carbohydrate ingestion. In: Van der Kamp JW, Jones J, McCleary B, Topping D (eds) Fibre: new frontiers for food and health. Wageningen Press, Wageningen, p 586
17. Pitino L, Randazzo CL, Mandalari G, Lo Curto A, Faulks RM, Le Marc Y, Bisignano C, Caggia C, Wickham MSJ (2010) Survival of *Lactobacillus rhamnosus* strains in the upper gastrointestinal tract. Food Microbiol 27(8):1121–1127. doi:10.1016/j.fm.2010.07.019
18. FAO/WHO (1998) Carbohydrates in human nutrition. Report of a joint FAO/WHO expert consultation. FAO Food Nutr Pap 66:1–140
19. Holm J, Lundquist I, Bjorck I, Eliasson A, Asp N (1988) Degree of starch gelatinization, digestion rate of starch in vitro, and metabolic response in rats. Am J Clin Nutr 47(6):1010–1016
20. Englyst HN, Quigley ME, Hudson GJ (1994) Determination of dietart fiber as non-starch polysaccharides with gas-liquid-chromatographic, high-performance liquid-chromatographic or spectrophotometric measurement of constituent sugars. Analyst 119(7):1497–1509
21. Kirsten WJ, Hesselius GU (1983) Rapid, automatic, high-capacity Dumas determination of nitrogen. Microchem J 28(4):529–547
22. Dionex (2004) Determination of unbound fat in various food matrices using accelerated solvent extraction (ASE)
23. Wickham MSJ, Faulks RM (2007) Apparatus, system and method. WO/2007/010238
24. Vardakou M, Mercuri A, Barker SA, Craig DQM, Faulks RM, Wickham MSJ (2011) Achieving antral grinding forces in

biorelevant in vitro models: comparing the USP dissolution apparatus II and the dynamic gastric model with human in vivo data. AAPS PharmSciTech 12:1–7
25. Lentner C (1981) Geigy scientific tables, vol 1. Units of measurement, body fluids, composition of the body, nutrition, 8th edn. Ciba-Geigy Basel, Switzerland
26. Franceschi VR, Krekling T, Berryman AA, Christiansen E (1998) Specialized phloem parenchyma cells in Norway spruce (Pinaceae) bark are an important site of defense reactions. Am J Bot 85(5):601–615
27. de Neergaard E (1997) Methods in botanical histopathology. Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen
28. Burger W, Burge MJ (2008) Digital image processing: an algorithmic introduction using java. Springer, Berlin
29. Wolever TMS, Miller JB (1995) Sugars and blood-glucose control. Am J Clin Nutr 62(1):S212–S227
30. Bantle JP, Laine DC, Castle GW, Thomas JW, Hoogwerf BJ, Goetz FC (1983) Postprandial glucose and insulin responses to meals containing different carbohydrates in normal and diabetic subjects. New Engl J Med 309(1):7–12
31. Press WH, Flannery BP, Teukolsky SA, Vetterling WT (1986) Numerical recipes, vol 547. Cambridge Univ Press, Cambridge
32. Wolever TMS, Brand-Miller JC, Abernethy J, Astrup A, Atkinson F, Axelsen M, Bjorck I, Brighenti F, Brown R, Brynes A, Casiraghi MC, Cazaubiel M, Dahlqvist L, Delport E, Denyer GS, Erba D, Frost G, Granfeldt Y, Hampton S, Hart VA, Hatonen KA, Henry CJ, Hertzler S, Hull S, Jerling J, Johnston KL, Lightowler H, Mann N, Morgan L, Panlasigui LN, Pelkman C, Perry T, Pfeiffer AFH, Pieters M, Ramdath DD, Ramsingh RT, Robert SD, Robinson C, Sarkkinen E, Scazzina F, Sison DCD, Sloth B, Staniforth J, Tapola N, Valsta LM, Verkooijen I, Weickert MO, Weseler AR, Wilkie P, Zhang J (2008) Measuring the glycemic index of foods: interlaboratory study. Am J Clin Nutr 87(1):247S–257S
33. Behme MT, Dupre J (1989) All bran vs cornflakes—plasma glucose and insulin responses in young females. Am J Clin Nutr 50(6):1240–1243
34. van Kempen T, Regmi PR, Matte JJ, Zijlstra RT (2010) In Vitro starch digestion kinetics, corrected for estimated gastric emptying, predict portal glucose appearance in pigs. J Nutr 140(7):1227–1233. doi:10.3945/jn.109.120584
35. Woda A, Mishellany-Dutour A, Batier L, François O, Meunier J, Reynaud B, Alric M, Peyron M (2010) Development and validation of a mastication simulator. J Biomech 43(9):1667–1673
36. Woolnough JW, Bird AR, Monro JA, Brennan CS (2010) The effect of a brief salivary α-amylase exposure during chewing on subsequent in vitro starch digestion curve profiles. Int J Mol Sci 11(8):2780–2790
37. Woolnough JW, Monro JA, Brennan CS, Bird AR (2008) Simulating human carbohydrate digestion in vitro: a review of methods and the need for standardisation. Int J Food Sci Technol 43(12):2245–2256. doi:10.1111/j.1365-2621.2008.01862.x
38. Germaine KA, Samman S, Fryirs CG, Griffiths PJ, Johnson SK, Quail KJ (2008) Comparison of in vitro starch digestibility methods for predicting the glycaemic index of grain foods. J Sci Food Agric 88(4):652–658. doi:10.1002/jsfa.3130
39. Englyst KN, Englyst HN, Hudson GJ, Cole TJ, Cummings JH (1999) Rapidly available glucose in foods: an in vitro measurement that reflects the glycemic response. Am J Clin Nutr 69(3):448–454
40. Gentilcore D, Chaikomin R, Jones KL, Russo A, Feinle-Bisset C, Wishart JM, Rayner CK, Horowitz M (2006) Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in Type 2 diabetes. J Clin Endocrinol Metab 91(6):2062–2067. doi:10.1210/jc.2005-2644
41. Granfeldt Y, Eliasson AC, Bjorck I (2000) An examination of the possibility of lowering the glycemic index of oat and barley flakes by minimal processing. J Nutr 130(9):2207–2214
42. Livesey G, Wilkinson JA, Roe M, Faulks R, Clark S, Brown JC, Kennedy H, Elia M (1995) Influence of the physical form of barley-grain on the digestion of its starch in the human small-intestine and implications for health. Am J Clin Nutr 61(1):75–81
43. Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Al-Sahab S, Bush D, Wright J, Fillery-Travis AJ (2000) Gastric response to increased meal viscosity assessed by echo-planar magnetic resonance imaging in humans. J Nutr 130(1):122–127
44. Marciani L, Gowland PA, Spiller RC, Manoj P, Moore RJ, Young P, Fillery-Travis AJ (2001) Effect of meal viscosity and nutrients on satiety, intragastric dilution, and emptying assessed by MRI. Am J Physiol Gastrointest Liver Physiol 280(6):G1227–G1233
45. Foster-Powell K, Holt SHA, Brand-Miller JC (2002) International table of glycemic index and glycemic load values: 2002. Am J Clin Nutr 76(1):5–56