

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

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1 **ABSTRACT**

2 **Purpose:** To identify the key parameters involved in cereal starch digestion and associated glycaemic response by
3 utilisation of a dynamic gastro-duodenal digestion model.

4 **Methods:** Potential plasma glucose loading curves for each meal were calculated and fitted to an exponential function.
5 The area under the curve (AUC) from 0–120 min and total digestible starch was used to calculate an *in vitro* glycaemic
6 index (GI) value normalised against white bread. Microscopy was additionally used to examine cereal samples
7 collected *in vitro* at different stages of gastric and duodenal digestion.

8 **Results:** Where *in vivo* GI data was available (4 out of 6 cereal meals) no significant difference was observed between
9 these values and the corresponding calculated *in vitro* GI value.

10 **Conclusion:** It is possible to simulate an *in vivo* glycaemic response for cereals when the gastric emptying rate
11 (duodenal loading) and kinetics of digestible starch hydrolysis in the duodenum are known.

12

13 **Keywords:** Starch, Glycaemic Index, Barley, Oat

14

1 INTRODUCTION

2 Cereal grains are the largest contributor to carbohydrate intake.[1] The only major source of glycaemic
3 carbohydrate is digestible starch.[2] Both the rate of production and rate of absorption of glucose in the duodenum
4 derived from starch, up to a maximum absorptive capacity of approximately 1g/min,[3] is largely dependent on the
5 amount of starch per unit time entering the duodenum from the stomach,[4] i.e. gastric emptying, and the accessibility
6 of that starch to pancreatic α -amylase.[5] The rate of gastric emptying is largely a function of the caloric density,
7 nutrient profile and volume of the ingested meal.[6] While pancreatic α -amylase is in excess and not rate limiting,[7] its
8 accessibility to starch is a function of the physical structure and chemical composition of the food entering the
9 duodenum.[5] The structure of food in the duodenum is, or may become, the rate limiting step in the production of
10 amylolysis products (oligosaccharides $>DP10$). Since these oligosaccharides are water soluble and highly mobile they
11 are rapidly hydrolysed into glucose by duodenal brush border membrane oligosaccharidases.[8,7] Glucose is then
12 rapidly and completely absorbed through the duodenum and transferred via the portal vein to the liver and then out into
13 the systemic circulation system.[9] The initial rate (0-120 min) of glucose appearance in the systemic circulation
14 system from fasting baseline is therefore a function of absorption from the duodenum. It roughly corresponds to a linear
15 increase to maximum followed by an exponential decay back to baseline [10,11] although the rate of absorption is an
16 individual variable depending upon age and gender.

17 This mechanism, referred to as duodenal glucose loading, induces a glycaemic response in the body in the
18 form of an excursion from baseline of plasma glucose. Excursion is a general function of glucose absorption minus
19 clearance, if glycogenesis, gluconeogenesis etc. are ignored. This excursion lasts the duration of loading of glucose
20 from the duodenum plus the time for plasma clearance ($T_{1/2} = 10-20$ min),[12,13] which although it is insulin
21 dependent, is mainly constant within an individual. Peak plasma glucose concentration therefore occurs during peak
22 loading at about 20-30 min after ingestion which is about the same time gastric emptying is complete for a breakfast
23 cereal meal of low energy load, or just after, since the entire bolus will then be subjected to amylolysis in the duodenum

24 With these observations in mind it should be theoretically possible to simulate the *in vitro* glycaemic response
25 of a cereal based meal and calculate an *in vitro* glycaemic index based on the kinetics of digestible starch hydrolysis in
26 the duodenal lumen attenuated to account for delivery within a 120 min period. Because the concept of GI is based on a
27 comparative system within an individual then there is an automatic assumption that glucose clearance is linear with
28 plasma glucose concentration. The only time this relationship becomes non-linear is when glucose is lost via the
29 kidneys. In fact, insulin response gives data sets which closely mimic those obtained by measurement of plasma glucose
30 [14]. There should therefore be no need (i.e. it is not necessary) to take into account, or correct for, all the preceding
31 individual physiological responses leading to intra- and inter-individual variation in a plasma glycaemic response as is
32 the case for two recently proposed alternative *in vitro* digestion models[15,16] that predict glycaemic response if one

1 just wants to determine an *in vitro* GI. Some of these variable physiological responses include differences in rates of
2 duodenal glucose absorption and plasma glucose disposal which in turn are individual variables based on insulin
3 production, insulin sensitivity, body mass, age, muscle glycogen stores to name just a few.

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

11

1 MATERIALS and METHODS

2

3 Grain processing

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

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

18

19 Degree of starch gelatinization

20 This was determined by measuring the heat of starch gelatinization (transition enthalpy ΔH) in industrially
21 processed samples by differential scanning calorimetry (DSC)[19] on a Mettler Toledo DSC823°. Milled cereal samples
22 (<0.5 mm) of ca. 30 mg were weighed into a 120 μ l stainless steel pan followed by the addition of 60 μ l water. Milled
23 pearled barley and oat groats from the same batch used to make the cereal samples were used as reference materials to
24 normalise against ungelatinized starch. The DSC scanning rate was 5 °C/min from 10-120 °C. An equivalent steel pan
25 containing silicon oil was used as reference. The instrument was calibrated prior to use against indium. ΔH was
26 calculated as the integrated area of the first peak in the thermogram per gram total starch (starch determined as
27 described below and expressed in terms of dry weight) using STAR SW 9.01 software along with gelatinization onset
28 temperature (T_o), peak temperature (T_p) and endset temperature (T_e).

29

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

31 Starch was determined by AACC standard method 76.13 (without the use of DMSO or ethanol wash) using a
32 Megazyme kit (Megazyme, Wicklow, Ireland). Non-starch polysaccharide (NSP) was determined by the method of

1 Englyst[20] using CRM 514 breakfast bran as a positive control. Mixed-linkage beta-glucan was via a Megazyme kit
2 according to AACC method 32.23.

4 **Determination of moisture, protein and fat**

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

9 ***In vitro* dynamic gastric digestion of test meals**

10 Test meals comprised 37.5g cereal product mixed with 200 g water. This provided a meal of approximately
11 590 kJ (140 kcal) for the oat meal and 558 kJ (133 kcal) for the barley meal. These values are typical of the
12 recommended breakfast cereal meal of around 35-40 g (approx. 135kcal, 567 kJ) depending on manufacturer. White
13 bread (70% extraction flour) corrected for water content and Kellogg's Cornflakes were used as control meals. Upon
14 addition of water the cereal meals were left to stand for 2 min to ensure constant hydration. The bread was used as
15 received. The meal was then eaten by one subject, but instead of swallowing the chewed boluses were spat out into a
16 beaker. Once all the meal had been chewed it was transferred to the stomach of the Dynamic Gastric Model (DGM)
17 already primed with 20 ml acidified salt solution (0.02 M HCl, 0.08 M NaCl, 0.03 M CaCl₂, and 0.9 mM NaH₂PO₄) for
18 simulated gastric processing. The simulated conditions were as for a normal fasted adult with physiological and
19 peripheral addition of acid and gastric enzymes, mixing, shear and residence time. Full details of the DGM which
20 include its design, its function, the method to prepare simulated gastric enzyme secretions, *in vitro* - *in vivo* validation
21 have been previously described[23,17,24]. The gastric process was started after a 1 min delay to allow the pH electrode
22 in the 'main body' of the DGM model to equilibrate. Initial starting conditions in the model were constant. The total
23 residence time of the meals was approximately 30 min during which samples of 30-40 ml of gastric digesta were ejected
24 from the antrum every 5-6 min. Samples were denoted G (gastric) 1-6 D (duodenal) 0 indicating no duodenal
25 processing.

26 Sample weight and pH were immediately recorded. The antral effluent was neutralised to pH 6.8 with
27 saturated sodium hydrogen carbonate and re-weighed. A 5g sub-sample was removed for simulated static duodenal
28 digestion, 25 ml for determination of viscosity using a TA Instruments AR 2000 with a cup and modified bob (vaned) at
29 shear rates between 0-1000 s⁻¹, and 1 ml fixed in 3 ml of 0.1M cacodylate buffer containing 3% glutaraldehyde and
30 stored at 4 °C for subsequent microscopic examination.

32 **Static duodenal digestion**

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

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

14

15 **Light Microscopy and image analysis**

16 The fixed digest suspension was vortex mixed for 10 seconds. Then 40-100 µl was pipetted out from the centre
17 of the suspension and spread out onto a glass microscope slide. These were dried overnight in a fume cupboard. The
18 slides were then mounted and examined under polarised light, in epifluorescence mode for autofluorescence with blue
19 light of 450-490 nm for excitation and a long band pass filter (>520 nm) used for visualisation of induced
20 fluorescence,[26] and after iodine-potassium iodide (IKI) staining.[27] All preparations were examined with a Leica
21 DMR light microscope and typical images photographed with a Leica DC3000 CCD camera. Image resolution was
22 2088x1550 pixels with a scale of 2.895 pixels/µm.

23 The visual texture of images at a magnification of x10 obtained from IKI stained digests of micronized barley
24 flakes and oat flour was assessed.[28] All images were processed and analyzed by image analysis software (Image J,
25 Wright, Toronto, Canada). The obtained digital images were divided into pixels (in our case 2088x 1550 pixels) of
26 which individual pixels take a value between 0 (black) and 255 (white) (8 bits grey level). Using the Isodata
27 algorithm[28] the pixels in the monochrome images were allocated values such that the iodine stained starch was
28 allocated black and the background white. The area fraction of IKI stained starch was calculated as a percentage of
29 pixels in the image that have been allocated black.

30

31 **Scanning Electron Microscopy (SEM)**

1 The fixed digest suspension was vortex mixed for 10 seconds. Then 300 μ l was pipetted out from the centre of
2 the suspension and transferred to a plastic tube covered at each end with microscope lens paper. This was submerged
3 under 0.05 M PIPES buffer, pH 7.2 followed by a graded series of alcohols for sample dehydration. Samples were then
4 dried in a critical-point dryer (BAL-TEC CPD 030) under CO₂, mounted on aluminium stubs and finally sputter coated
5 (Polaron SC 7640) twice with gold-palladium. The samples were viewed in a Zeiss EVO-50-EP SEM.

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

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

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

28 ***In vivo* GI measurements of barley test meals**

29 These measurements were conducted by Leatherhead Food Research, Leatherhead, Surrey, UK and followed
30 the FAO/WHO protocol[18] . Twelve healthy subjects between 18-65 years of age took part in the study. Informed
31 consent was obtained from each individual. Each subject had a similar meal prior to each morning test session. The test
32 meal and glucose reference meal were tested in each subject in duplicate and triplicate respectively. The study was

1 randomised to remove any order effects. Each subject received one portion of a test meal containing 25 g available
2 carbohydrate in which the whole amount was consumed in a 15 minute period. This smaller amount of available
3 carbohydrate, rather than the standard 50g, was necessary for each meal so they would be manageable to consume
4 without milk. The two meals comprised 46 g micronized barley flakes or 41 g extruded barley flakes in 200 ml water.
5 The glucose reference meal consisted of 25 g glucose dissolved in 250 ml water. After starting the consumption of the
6 meal (T=0) blood samples were taken at 15min intervals for the first hour and then at 30 min intervals up to 2 hours.
7 Blood was collected into small tubes containing lithium-heparin following a finger-prick. Tubes were immediately
8 mixed and centrifuged at 3000 rpm for 10 min to separate the plasma. Blood glucose was then measured via a YSI 2300
9 Stat Plus Glucose and Lactate Analyser. Data analysis was analysed by the standard WHO/FAO protocol.[18] The GI
10 calculated from the glucose reference (\pm SEM) was converted to a white bread reference GI by dividing the glucose
11 reference result by 0.71.[32]

12

13 **Statistical comparison of GI values**

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

1 RESULTS

2

3 Characteristics of cereal products

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

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

20

21 Viscosity of antral digesta and antral pH

22 The viscosity of the digesta ejected from the antrum (entering the duodenum) was measured between 1 and
23 500 s⁻¹. The viscosity at a nominal antral shear rate of 26 s⁻¹ was taken to be the viscosity experienced in the antrum.
24 For all cereal meals and gastric emptying times the viscosity was generally low and less than 2 Pa·s. The viscosity of
25 antral digesta from the micronized barley flake meal decreased from over 2 to <0.01 Pa·s during the course of digestion
26 (Fig. 1(a)). The initial antral digesta viscosity of the oat flour meal was an order of magnitude smaller and by the latter
27 stages of gastric digestion it was similar to the barley flake meal (Fig. 1(a)). Antral digesta derived from the extruded
28 barley flake meal had a slightly greater viscosity than the oat flour in the early stages of digestion. The digesta viscosity
29 then slightly increased before steadily decreasing, but to a level slightly higher than the oat and barley samples not
30 subjected to extrusion (Fig. 1(a)). The viscosity of the antral digesta derived from the extruded barley flake meal
31 showed a similar trend to that of extruded oat meal although the viscosities were roughly double (Fig. 1(a)). Similarly in
32 the later stages of digestion the antral digesta viscosity of extruded barley was slightly greater (ca. 0.3 Pa·s) than those

1 of the micronised barley flake meal at corresponding times of gastric emptying (Fig. 1(a)). The viscosity of the first
2 antral digesta from the white bread meal was 0.1 Pa·s which was lower than the corresponding oat and barley antral
3 samples. In the next two subsequent samples the viscosity increased to just under 0.5 Pa·s and in the last two samples
4 decreased to just less than that of the first gastric digesta. The antral viscosity of the cornflake meals was more constant
5 throughout digestion at between 0.11-0.23 Pa·s.

6 In terms of antral pH this varied from 3.9-4.5 in the first 16 minutes of gastric digestion for the micronised
7 barley flake meal before progressively dropping to 1.8 towards the end of gastric processing (Fig. 1(b)). A similar trend
8 in antral pH was observed for the other oat, barley and white bread meals with an initial higher pH up to a maximum of
9 5.5-6 followed by an eventual decline in pH to around 2 as gastric digestion approached completion (Fig. 1(b)). It
10 seemed cornflakes had a much lower initial pH buffering capacity in the antrum compared to other samples with pH
11 only reaching a maximum of about 3.6 after 16 min processing before dropping back to about pH 2 towards the end of
12 gastric processing (Fig. 1(b)).

13

14 **Microscopy examination of antral digesta and duodenal digestion products**

15 Antral digesta examined by light microscopy from all barley and oat meals contained various sizes (ca. 0.01-1 mm²) of
16 bran fragments. These have a blue/turquoise autofluorescence as typified by bits of aleurone (Fig. 2(a-b)). Oat samples
17 contained smaller bran fragments than the barley samples although there appeared to be no major differences between
18 extruded and non-extruded samples or as a function of digestion time.

19 Light microscopy examination also revealed an abundance of small and large round and elliptical barley starch
20 granules in the micronised barley flake meal entering the gastric compartment and in the first gastric digesta collected
21 between 1-16 min (Fig. 2(c-d)). They gradually decrease in numbers (Fig. 3(a)) as a function of increasing gastric
22 digestion time. Most of these starch granules are absent after 27-33 min in the gastric compartment (Fig. 2(e-f)). While
23 intact starch granules dominate in the first gastric sample with only a 6 min gastric digestion time (Fig. 4(a)), eroded
24 and pitted granules increase in abundance in subsequent gastric samples (Fig. 4(b-c)) until only the bran fragments
25 dominate in the material subject to the longest gastric digestion time (Fig. 4(d)). Changes in the abundance and
26 appearance of small and large barley starch granules as a function of digestion time in the duodenum are much less
27 pronounced (Fig. 2(g-h), Fig. 3(a), Fig. 4(e-f)) and they look similar to the gastric sample they originated from.

28 In the simulated digestion of the oat flour meal most oat starch granules occur as single granules or in clusters
29 (Fig. 2(i-j)). In contrast to the micronised barley flake meal there is an abundance of starch granules in all the gastric
30 digesta emptied from the stomach (Fig. 2(j), Fig. 3(b)). However, it seems that the starch granules in the part of the
31 meal that underwent the longest gastric digestion are more susceptible to further digestion in the duodenum (Fig. 2(k),

1 Fig. 4(b)) than starch granules that pass out of the gastric compartment at a much earlier stage (Fig. 2(l), Fig. 4(b)). Oat
2 starch granule clusters become less frequent as a function of digestion time.

3 The starch present in the extruded barley and oat is visualised in the first gastric emptying as dense blue
4 structure-less clumps (Fig. 2(m-n)). The clumps become less dense and more disperse as gastric digestion proceeds
5 (Figure 2(o-p)) for both meal types. Further starch digestion occurs in the duodenum (Fig. 2(q, r, s, t) but it does not go
6 to completion and some starch remains.

7

8 **Glycaemic response and index**

9 When white bread was used as the reference meal there was no significant difference ($p < 0.05$) between the
10 calculated *in vitro* GI values and corresponding *in vivo* GI values (based on 120 min AUC and equivalent available
11 carbohydrate content) for cornflakes and the two barley products (Table 4). Compared with the other test meals studied
12 *in vitro* the micronised barley flake meal had a low digestible starch content, peak rate of starch hydrolysis and a long
13 duration of starch digestion (Table 4, Fig. 5(b)). This resulted in an extended and flattened potential plasma glycaemic
14 loading curve (Fig. 5(b)). The *in vitro* calculated GI was 66 and quite similar to that calculated *in vivo* (Table 4). The
15 peak of the GI curve in plasma *in vivo* (Fig. 6) occurs around 30 min. Even though the fitted peak rate of starch
16 hydrolysis occurs much later at 72 min (Table 4) the rate is similar to that at 30 min (Fig. 6). The decline in plasma
17 glucose seen in the glycaemic response curve *in vivo* occurs gradually from 30 min to beyond 150 min which is similar
18 to that *in vitro*.

19 At the other extreme the cornflake and white bread meals had over double the content of digestible starch than
20 the micronised barley flake meal and much greater peak starch hydrolysis rates of 462 and 346 $\text{mg}\cdot\text{min}^{-1}$ respectively
21 (Table 4). This occurred within in a much shorter hydrolysis time of 112 and 154 minutes respectively. Consequently
22 the *in vitro* potential plasma glycaemic loading curves are sharp and narrow (Fig. 5) and the *in vitro* GI similarly high at
23 111 for cornflakes (Table 4). The plasma glucose curves for these two meals examined from the literature [33,14]
24 closely resemble the *in vitro* potential plasma glycaemic loading curves (Fig. 5(b)). As for the micronised barley flake
25 meal peak plasma glucose occurs at 30 min while the fitted rate occurred at 47 min. For the cornflake meal the plasma
26 glucose then rapidly declines to baseline by 90 min while for the white bread meal the decline is more gradual and
27 protracted with a return to baseline >120 min [33,14]. A similar trend is observed *in vitro* (Fig. 5).

28 For the oat flour meal the total digestible starch content was less than the white bread and cornflakes meals.
29 But even though the duration of *in vitro* starch hydrolysis was similar to the micronised barley flake meal the GI was
30 much higher (Fig. 5(c), Table 4). These higher values reflect a quite rapid rate of starch hydrolysis in the meal as well as
31 the greater availability of total digestible starch. In contrast the extruded barley and oat flake meals contain almost half
32 the total digestible starch content than the oat flour meal. But because this starch is rapidly digested (Fig. 5) the *in vitro*

1 GI values are high at 103 and 105 respectively. It is no surprise that the *in vitro* potential plasma glycaemic loading
2 curves look similar to each other (Fig. 5(a)) and are slightly right skewed (Fig. 5). This pattern is reflected in the plasma
3 glucose curve for the extruded barley meal (Fig. 6) where there is a shorter but greater excursion of plasma glucose
4 ending at about 90-100 min. The maximum rate of loading *in vitro* for the extruded barley meal also coincides with the
5 plasma glucose peak maximum in the *in vivo* GI curve (Fig. 6).

6

1 DISCUSSION

2

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

12 Our results also show that it is sufficient only one individual chews the meal and expectorates it just prior to
13 swallowing. This is because there is no significant inter-individual variability in the particle size of food boluses at the
14 end of chewing[35] and the contribution of salivary α -amylase to plasma glucose loading is rapidly nullified by the
15 action of pancreatic α -amylase in the duodenum. [36] Furthermore, when *in vivo* GI is determined by a panel of
16 volunteers it is independent of the person chewing since: (1) it is a comparison within an individual (2) it is a mean
17 across a number of panellists. We demonstrate that the DGM and associated duodenal model provide data sets which
18 can be used to describe the GI of some cereals. Most importantly, GI is a comparative indexation method where there is
19 a standard (100%) against which all others are judged. Each subject in a volunteer panel therefore provides a data set for
20 the standard and the test material and the two AUC's for plasma glucose are compared within an individual (mastication
21 and gastric emptying differences are nullified). Relative GI's are then averaged across the panel. The fact that only one
22 person chewed the food is no different to the contribution of a single individual to a panel where the mean data is
23 derived.

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

30 A whole host of other *in vitro* carbohydrate digestion models exist that can predict the glycaemic properties of
31 foods[37] but they do not simulate the glycaemic response over time and seldom accurately replicate the biological
32 digestive processes in the human especially with respect to dynamic gastric processing. They often only predict a GI

1 value based on chemically operational definitions [37,38] such as in the concept of rapidly and slowly digestible
2 starch.[39,1] In order to predict GI one really needs to take into account the rate of gastric emptying in addition to the
3 total load of available carbohydrate. Apart from at breakfast, carbohydrates are usually consumed as part of a complex
4 meal. In the postprandial state where the other components of the meal, particularly fat [40], have an impact on the rate
5 of gastric emptying the standard concept of GI becomes meaningless. The glycaemic response under such
6 circumstances is overwhelmingly dictated by the rate of gastric emptying rather than any changes in the accessibility or
7 digestibility of starch. As a consequence it is possible to convert a high GI food into a low GI food, not by changing the
8 inherent digestibility of the starch in the duodenum, but simply by reducing the rate of delivery from the stomach.

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

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

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

31 Whereas the bolus formed upon chewing and swallowing of the other processed and readily hydrated cereal
32 meals is a soft and a easily dispersed slurry of small particles (<2mm) the bolus formed upon swallowing the

1 micronised barley flake meal was more heterogeneous and also comprised large botanically intact cereal pieces. This
2 has been noted earlier in barley flake meal samples collected from ileostomy subjects.[42] In the antrum the swallowed
3 food is subjected to an element of selective sieving. Accordingly the largest particles (>2 mm) tend to be retained until
4 the latter phase of gastric emptying. Larger pieces remaining in the antrum after gastric processing are eventually
5 emptied by the 'housekeeper' migrating motor complex, type III wave during fasting. However sieving does not explain
6 the reduction of starch granules in the digesta of the micronised barley flake meal collected from the antrum as a
7 function of gastric emptying.

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

19 Although some salivary α -amylase retains activity in the stomach it is thought to be somewhat diminished
20 because α -amylase exposed to gastric secretions is rapidly degraded. Hence it is traditionally thought that salivary α -
21 amylase plays a minor role in gastric starch hydrolysis[7] yet this still may be enough to account for the pits observed
22 on some starch granules in digesta with the longest gastric residence time. Other buccal enzymes, such as lingual lipase,
23 that are inactivated by low pH can retain their activity for up to 1 h in the centre of the bolus because gastric contents
24 are heterogeneous and often poorly mixed and therefore shielded from contact with the gastric secretions.[44] Perhaps
25 in a similar manner α -amylase also remained active in parts of the micronised barley flake meal. At present this
26 explanation is still somewhat speculative.

27 To ultimately confirm or refute some of the possible interpretations of the results from this study it is quite
28 clear more replicate experiments with different and more complex meals, for example adding milk to cereals, are
29 required. The results and approach used here also support and reinforce extensive earlier validation of the DGM against
30 the typical *in vivo* state. Furthermore, and assuming the *in vivo* state is represented accurately, one can start to begin to
31 understand the key processes that are involved in an associated biological response to an ingested food or meal. Some
32 of these biological processes in relation to starch digestion in some simple cereal meals have been investigated and

1 discussed in this study and present some new avenues for future research into the understanding the role of starch
2 digestion in health and disease. As a method of determining GI of foods it is currently too premature to propose our
3 model as a viable alternative to standard *in vivo* GI testing. What is proposed here is an approach that needs further
4 investigation and refinement.

5

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Figure Legends

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

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).

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

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).

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 (\pm 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.

TABLES

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

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

	White bread*	Cornflakes*	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

Table 2. Main nutritional composition of cereal products (% fresh weight). *Values from[2].

	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

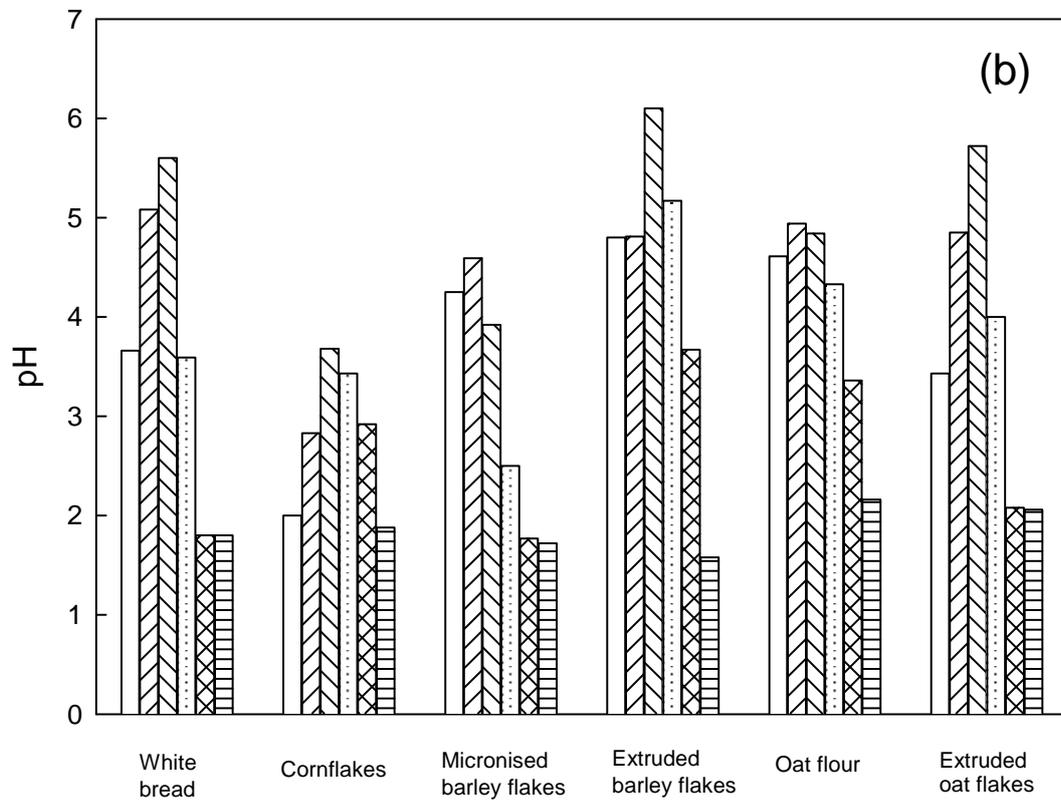
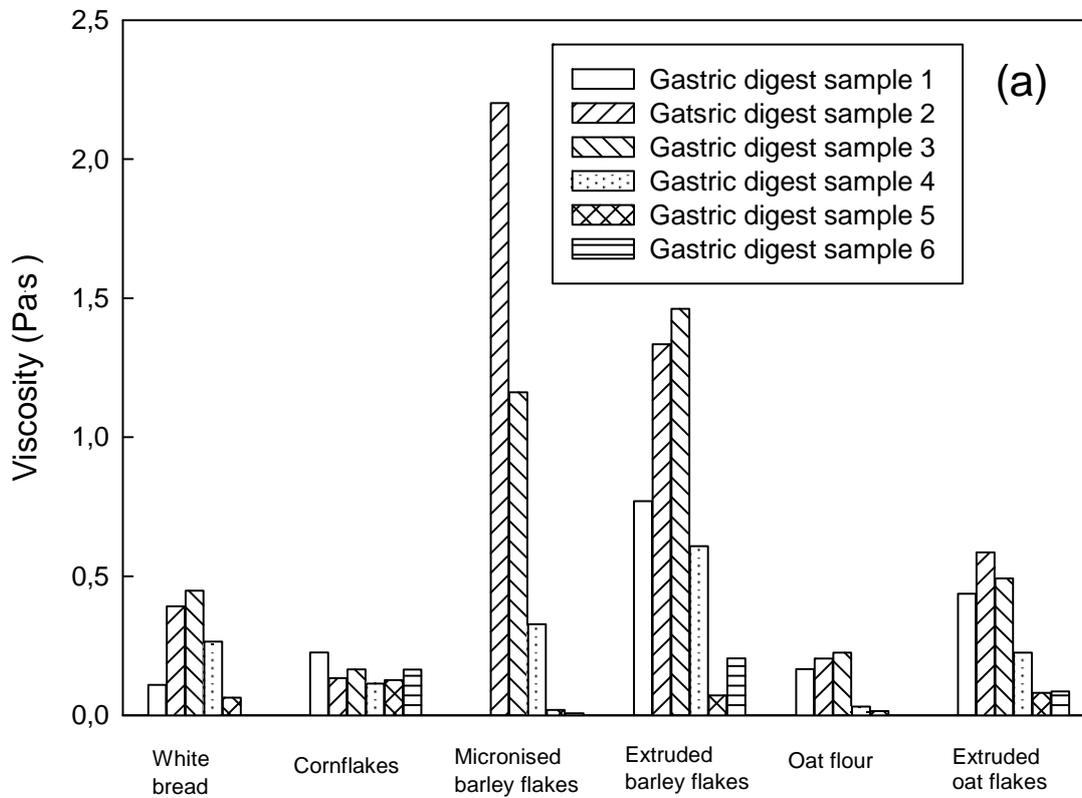
Table 3 Mean (n=2), onset (T_o), peak (T_p), conclusion (T_c) temperature, enthalpy of gelatinisation (ΔH) and degree of gelatinization of industrially processed oat and barley products.

Food	Peak rate (mgmin ⁻¹)	Peak Time (min)	Total digestible starch (g)	<i>In vitro</i> GI	<i>In vivo</i> GI*	
					Mean	SEM
White Bread	346	54	28.1	100	100	
Cornflakes	462	47	28.7	118	†116	‡5
Oat Flour	221	59	20.5	92	N.D.	
Extruded barley flakes	205	37	12.6	103	96.6	‡7
Micronised barley flakes	92	72	12.8	66	70	‡6
Extruded oat flakes	204	39	11.1	105	N.D.	

Table 4 *In vitro* modelled characteristics of starch hydrolysis for 6 cereal meals. N.D. = not done. *According to FAO/WHO protocol[18]. †Value from an international table of glycaemic index. Mean SEM of 5 studies[45]. ‡ SEM of 1 study n=12.

FIGURES

Figure 1



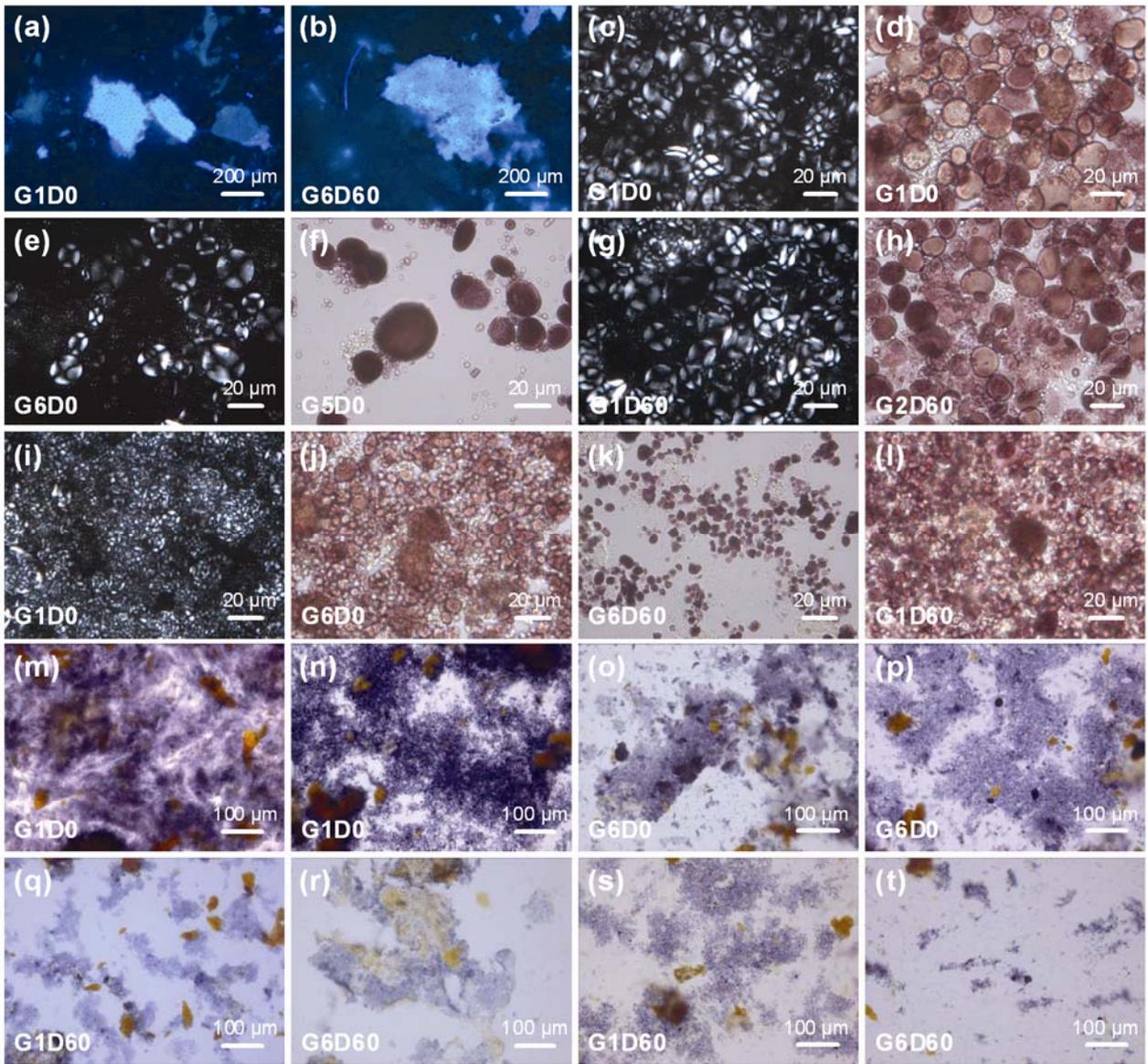


Figure 2

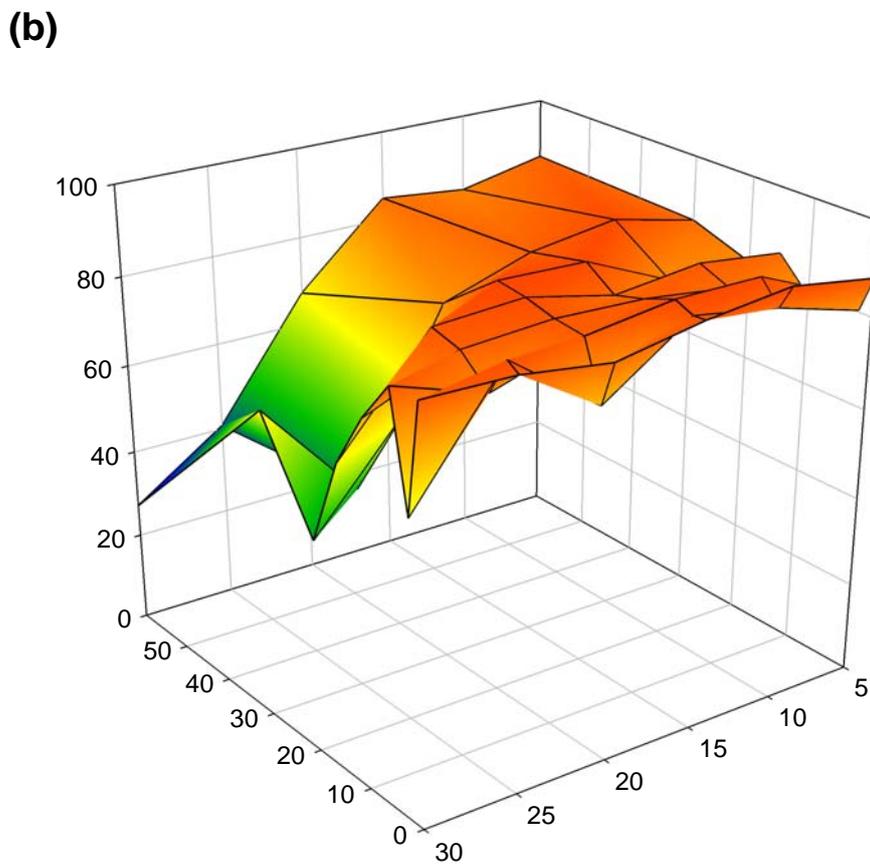
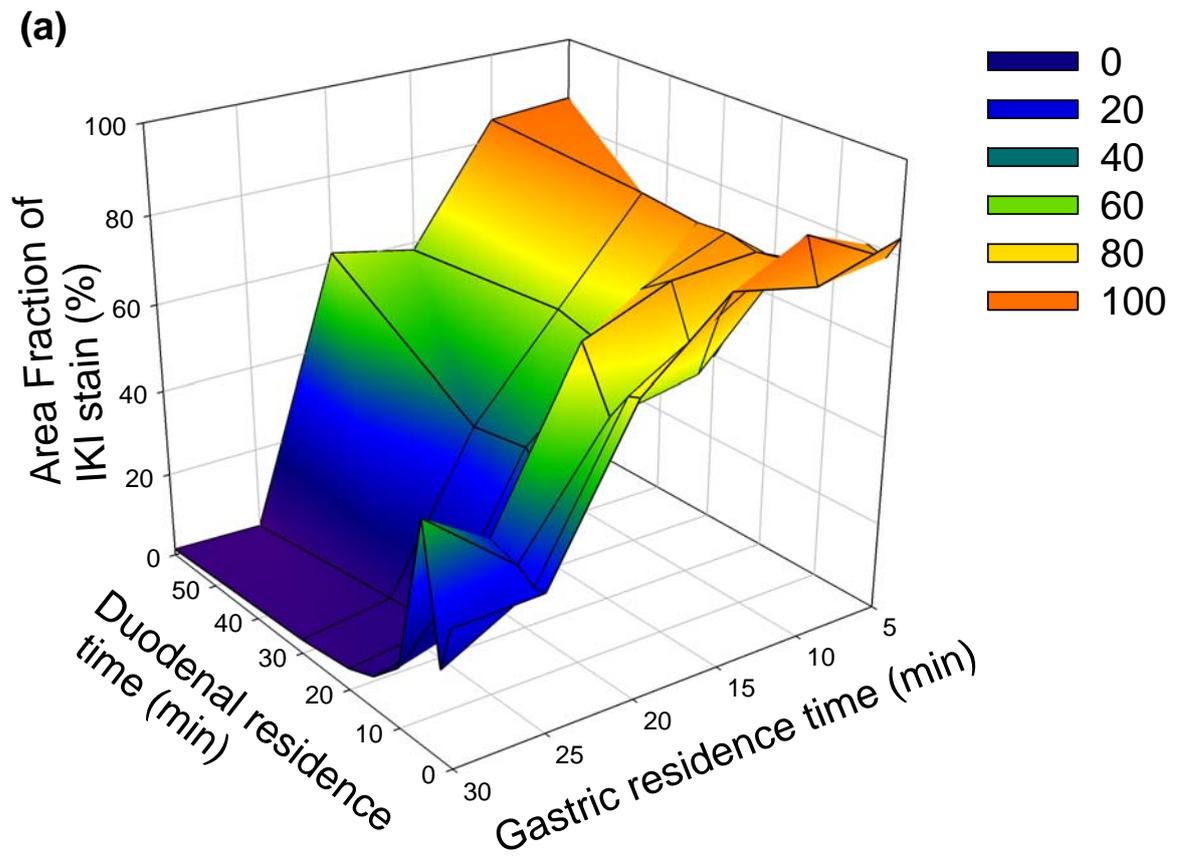


Figure 3

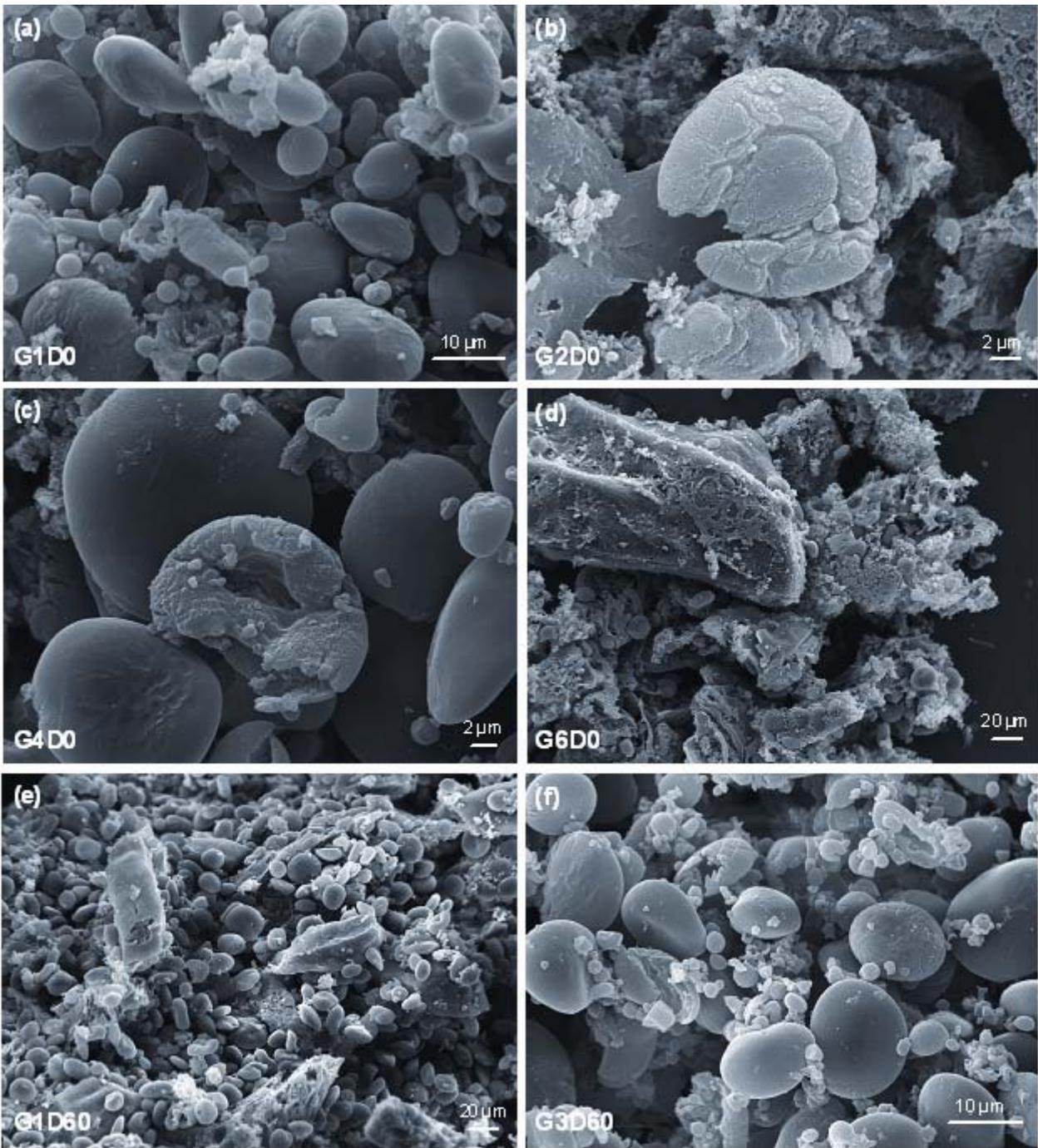


Figure 4

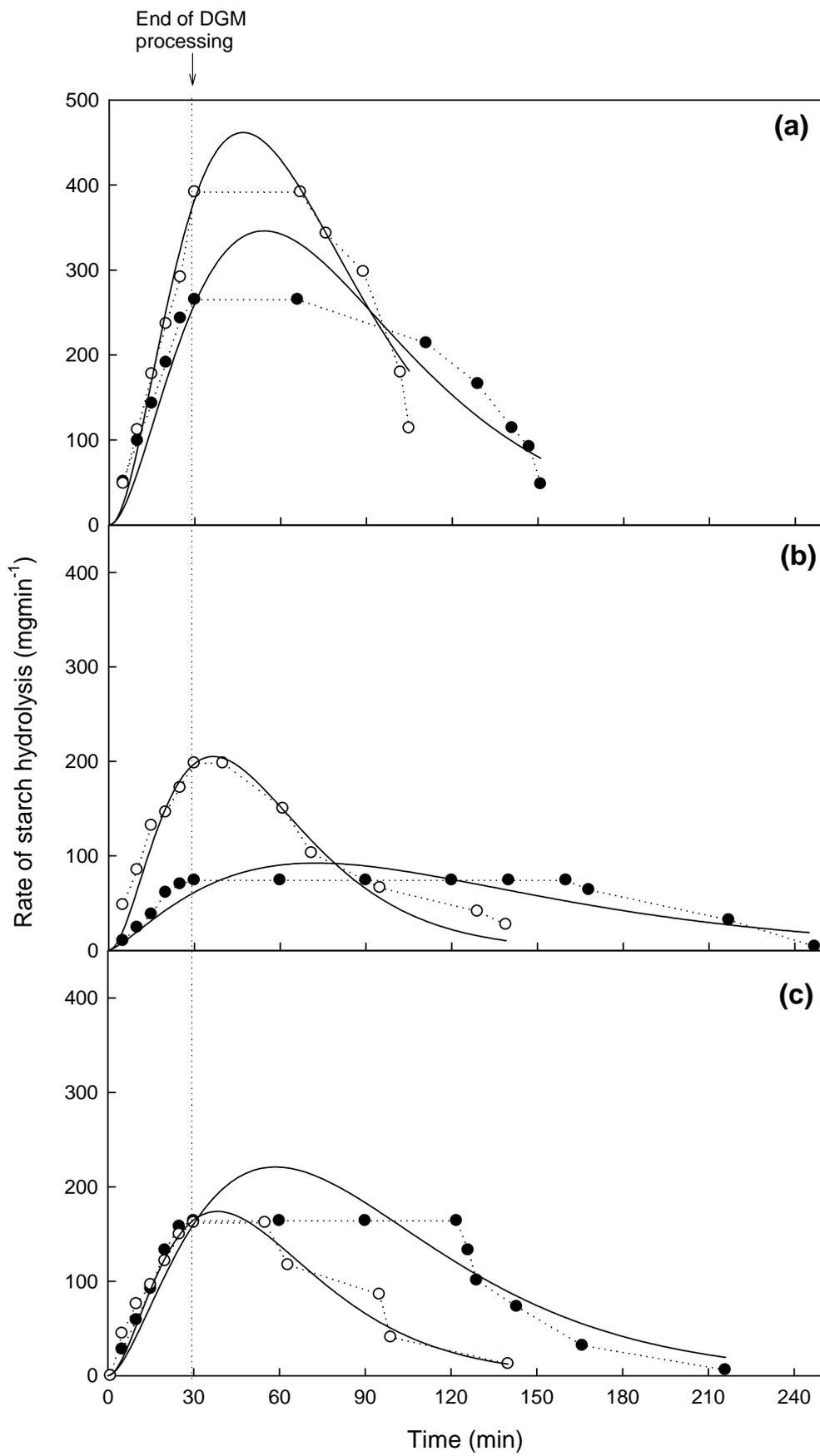


Figure 5

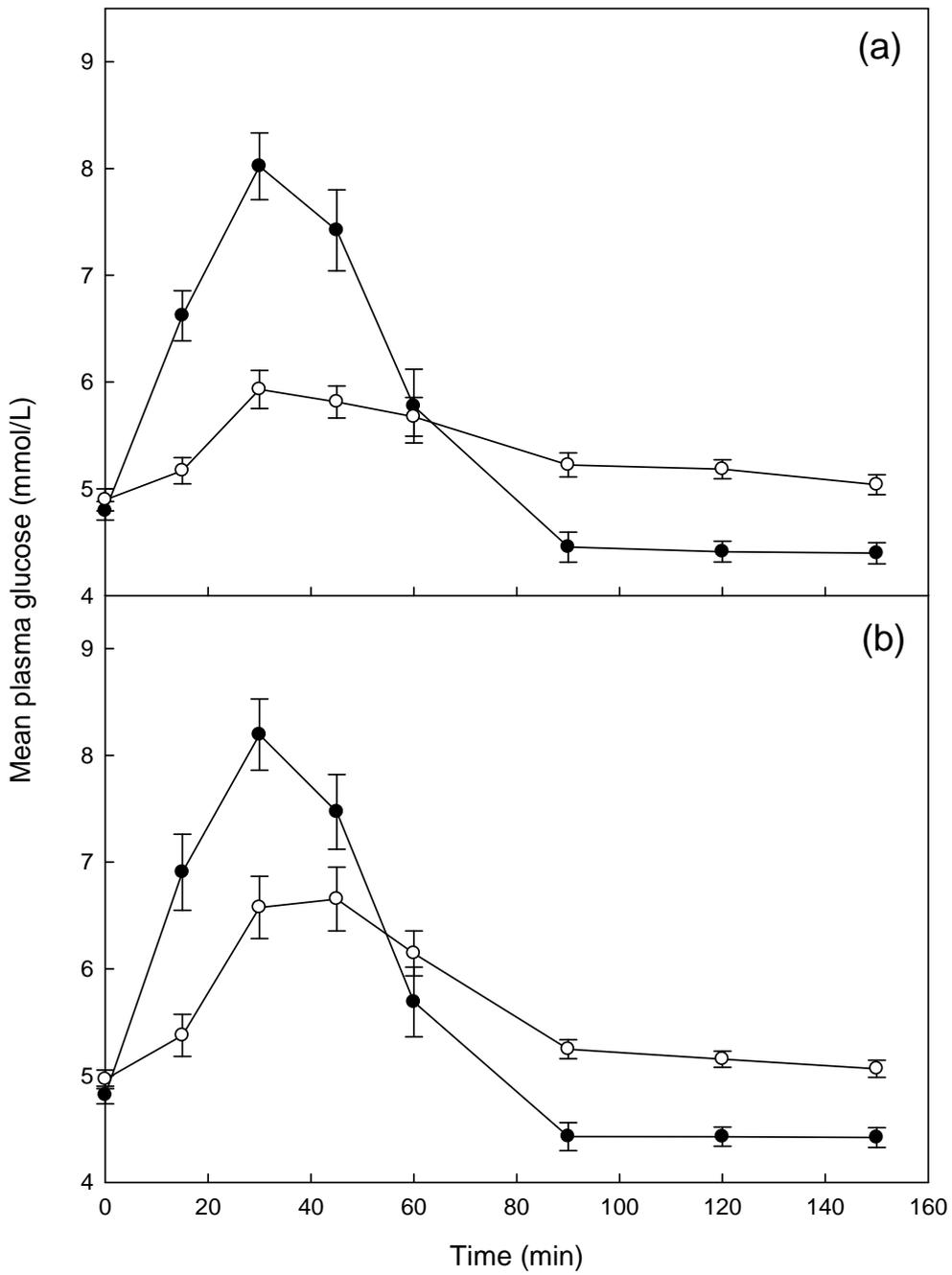


Figure 6