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High-amylose wheat lowers the postprandial glycemic response to bread in healthy adults

A report for Arista Cereal Technologies

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Executive summary

This report describes a randomised, double-blinded, crossover controlled study that determined whether breads made from High Amylose Wheat (HAW) dampen postprandial glycemia compared to conventional wheat (Low Amylose Wheat, LAW) breads.

Replacing conventional with high amylose wheat flour is an effective strategy for lowering postprandial glycemic and insulinemic responses to bread.

Substitution of refined flour for wholemeal, had no effect on glycemia or any of the other metabolic endpoints measured.

The amylose contents of the breads did not affect markers of satiety.

Further benefit from using high amylose flour in other wheat based food products such as ready-to-eat breakfast cereals and bars, noodles and doughs may result in the lowering of the glycemic impact of a range of different foods and deserves further investigation.

1 Background

Diet-related chronic diseases, such as coronary heart disease and diabetes, are major causes of morbidity and mortality in both affluent industrialized countries and emerging nations. Increased consumption of whole-grain cereal foods is recognised as an important approach for reducing the risk of these prevalent health problems (Koh-Banerjee et al. 2004, Richardson 2003, Slavin 2000, Venn and Mann 2004). These benefits of whole grains are largely attributed to their dietary fibre component of whole-grains which includes non-starch polysaccharides (NSP) and resistant starch (RS).

Although people are eating more wholegrains, refined starches from major cereals such as wheat, rice and corn that elicit a high glycemic response continue to be the major forms of carbohydrates consumed across the globe. To address this, corn and barley varieties and food ingredients have been developed that contain elevated RS levels by increasing the proportion of amylose in the starch. Foods made from these high amylose cereals induce lower postprandial glycaemic responses than foods made from conventional grains (Akerberg et al. 1998, Al-Tamimi et al. 2010, Aldughpassi et al. 2012, Behall and Hallfrisch 2002, Behall and Scholfield 2005, Goddard et al. 1984, Granfeldt et al. 1995, Granfeldt et al. 1994, Granfeldt et al. 1993, King et al. 2008, Klosterbuer et al. 2012, Miller et al. 1992). However, for most cereal-based processed products, such as bread, pasta, and noodles, which supply ~20% of the food calories for the world population, wheat is the preferred base flour and typically contains low levels of RS. Wheat starch contains low levels of amylose, typically 25 -30%, but can be as high as 38% (Yamamori and Endo 1996). However, bread made from wheat with a slightly higher level of amylose showed a similar glycemic and insulinemic response to that of conventional bread (Hallstrom et al. 2011). A wheat variety containing double this level of amylose (85%) has been developed by our group (Regina et al. 2015), however its glycemic and metabolic impact was not known.

The primary aim of this study was to determine whether breads made from a newly developed variety of wheat containing an elevated level of RS dampens postprandial glycemia. Breads made from the wholemeal and refined white flours were used to ascertain the effect of flour processing on glycemic response. To evaluate changes in glycemic response incretin hormones were measured during the postprandial period. A visual analogue scale was also used to evaluate feelings of fullness and cravings, along with a hormonal indicator of satiety (plasma ghrelin). Breath hydrogen levels were also monitored in a subset of individuals to assess changes in the magnitude and duration of fermentation following test bread consumption.

2 Methods

2.1 Study population

Twenty volunteers (15 women, 5 men) with a mean age of 30 ± 2 y and body mass index (BMI) of 23 ± 0.6 kg/m^2 were included in the study (Table 1). Inclusion criteria were as follows: aged 18-65 years, BMI 18.5 - ≤ 27.5 kg/m^2 and a normal fasting blood glucose concentration of 3.5 – 5.5 mmol/L. The exclusion criteria were as follows: known presence of diabetes, smoker, pregnant or lactating, sufferer of bleeding disorders, known food allergy, hypersensitivity or intolerance to wheat and/or starchy foods, taking medications known to influence glucose tolerance or gastric emptying (oral contraceptives are excepted), persons considered by the investigator to be unwilling, unlikely or unable to comprehend or comply with the study protocol, participation in another research study within 30 days preceding the start of this study, known history or presence of gastrointestinal, renal or hepatic disease of any cause, night shift workers. Participants provided written, informed consent to the study protocol approved by the CSIRO Human Ethics Committee. This study was registered at anzctr.org.au as ACTRN12616001289404 on 14/09/2016 and was conducted between October and December 2016.

Table 1 Baseline participant characteristics[1]

	Participants (n=19)
Age, y	30 ± 2
Sex, n (%)	
F	15 (75%)
M	5 (25%)
Body weight and composition	
Body weight, kg	67 ± 3
BMI, kg/m^2	23 ± 0.6
Fasting glucose, mmol/L	4.6 ± 0.4
Cardiovascular disease risk markers	
Total cholesterol, mmol/L	4.8 ± 0.2
LDL cholesterol, mmol/L	0.9 ± 0.1
HDL cholesterol, mmol/L	1.5 ± 0.1
Triglycerides, mmol/L	2.8 ± 0.2
Non-esterified fatty acids, mmol/L	0.4 ± 0.1
Habitual diet (24 h recall)	
Total energy, kJ/d	8046 ± 268
Protein, g/d	91 ± 11
Fat, g/d	80 ± 11
Carbohydrate, g/d	197 ± 17
Sugars, g/d	79 ± 8
Starch, g/d	118 ± 13
Dietary fibre, g/d	24 ± 2
Refined grain serves, number/d	4.5 ± 0.8
Wholegrain serves, number/d	1.7 ± 0.4
Wholegrains:refined grains, %	27 ± 7

[1]To convert mmol/L to mg/dL, multiply by 18 (for glucose), 38.7 (for cholesterol), and 88.6 (for triglycerides). Mean ± SEM (all such values).

2.2 Recruitment and screening

The participants were recruited from the CSIRO Biosecurity and Health Research database and the CSIRO website. To facilitate compliance, participants were provided with gift vouchers on the completion of the study to an amount corresponding to time spent in the study.

Any volunteer who responded to the study advertisements via e-mail or a telephone call was contacted to determine further interest in study participation. Volunteers were provided with information about the study design and, if interested, a first screening telephone questionnaire was administered to determine general eligibility. If eligibility was established, a pre-screening appointment was scheduled to acquaint them with study procedures and establish whether their fasting blood glucose level was in the normal range (3.5 – 5.5 mmol/L). If a potential participant's characteristics fell within the predetermined criteria, the individual was invited to commence the study. Seventy-three volunteers were screened, and 20 participants were enrolled in the study (Figure 1). One volunteer withdrew on the first visit of the study because they would not allow the nurse to insert the intravenous cannula into their forearm for blood collection.

2.3 Study design and intervention

A single-centre, randomised, double-blinded, crossover controlled study that involved seven food challenges was conducted over seven consecutive weeks. Three of the challenges consisted of a 300 mL glucose drink (Carbotest, Lomb Scientific) that contained 50 g glucose and was consumed on visits 1, 4 and 7. On visits 2, 3, 5 and 6 there were four bread challenges. The order of test breads were randomly allocated to each individual using a Latin square randomisation sequence which included four unique sequences; ABDC, BCAD, CDBA and DACB. The participants were instructed to maintain their dietary habits and daily routine for the duration of study (approximately 8 wk), avoiding eating foods high in fibre on the evening prior to testing and also to avoid heavy exercise on the day before and morning of each test. Consumption of alcohol was restricted on the day prior to testing.

Participants and clinic staff were blinded to the composition of each test bread and the test breads were only decoded once data clean up and preliminary statistical analysis were completed.

The four different wholemeal or refined wheat breads were made from conventional or high amylose wheat flour using a standard bread recipe. The flour and others ingredients (water, oil, sugar, salt, yeast and improver) were mixed at low speed for 3 min then at high speed for 7 min in a spiral kneader (Diosna, Osnabrück, Germany). After 15 min at ambient temperature, doughs were divided into 560g portions, then shaped, placed into a pan and proofed at 38°C with 80% humidity for 50 min. Breads were baked at 220°C for 37 min in a retail oven then allowed to cool for 2 hr before they were packaged. The breads were made by Limagrain Cereal Ingredients in France and shipped to Australia at -20°C. On the morning

of each test day, the breads were thawed by placing them at room temperature, the crust was removed and the bread was portioned to the designated serve size of 121 g. Samples of each test bread were analysed for starch, sugar, total dietary fibre, fat and protein content (Table 2). Freeze-dried and milled samples of each test bread were analysed in duplicate using standard Association of the Official Analytical Chemists (AOAC) methods.

Table 2 Energy and nutrient composition of the LAW and HAW breads[1]

	LAW Refined flour	LAW wholemeal	HAW Refined flour	HAW wholemeal
		g/100g		
Moisture	37.0	37.3	44.0	43.4
Fat	3.4	3.7	2.8	3.6
Protein	10.8	12.1	13.1	15.2
Ash	1.7	2.2	1.5	2.1
Fibre	3.3	8.2	5.5	10.4
Starch	35.2	26.9	28.5	19.9
Sugars	6.1	6.0	5.0	5.2
Resistant starch	0.4	0.3	4.7	3.2
		kJ/100g		
Energy	1045	984	951	922

[1] Resistant starch was measured by M^{c}Cleary method. Energy content of bread determined using energy values for protein (17 kJ/g), fat (37 kJ/g), carbohydrate (17 kJ/g) and total fibre (10 kJ/g).

At the first visit participant height was measured using a stadiometer (SECA, Hamburg, Germany), body weight was measured using calibrated electronic digital scales (AND HW-PW200 Scales) and BMI was calculated using the formula: weight (kg)/height2 (m2). A 12 hr fasted blood glucose concentration was determined in a finger prick blood sample by HemoQue. Blood was also collected from the antecubital vein and total cholesterol, LDL and HDL cholesterol, total triglyceride and free fatty acid concentration were determined. During the week of the first visit, the habitual diets and energy intakes of the participants were determined using a 24 hr food recall conducted by a dietitian via a phone interview.

On the morning of each meal test, participants arrived at the clinic following an overnight fast, and an intravenous cannula was inserted in an antecubital vein and a fasting blood sample withdrawn. Participants were instructed to consume the test meals within 15 min along with up to 250 mL of water (same water volume provided at each visit). Following the commencement of eating, sequential blood samples were collected at designated intervals

(15, 30, 45, 60, 90, 120, 150, 180 min) via the indwelling cannula in the following tubes; sodium fluoride coated tubes (Vacuette, Greiner bio-one, Austria) for measurement of glucose, P800 tubes (BD Biosciences, North Ryde, Australia) for measurement of insulin, GIP, active GLP-1, ghrelin and PYY and serum tubes (Vacuette, Greiner bio-one, Austria) for measurement of nitrotyrosine and I-CAM-1. Perceived appetite, satiety and mood were assessed using a visual analogue scale which was completed by participants at 30 min intervals for 3 h from baseline. Following completion of the sampling phase, the cannula was removed and participants were provided with light refreshments prior to leaving the Clinical Research Unit.

Six of the 20 subjects were randomly selected to also provide breath samples so that breath hydrogen levels could be determined (4 test breads only) using a Gastro+ Gastrolyser (Bedfont, Rochester, England). Breath hydrogen was measured at baseline and at 30 min intervals during the blood sampling period (0-180 min). Once the last blood sample was collected at 3 hr, participants continued to measure their breath hydrogen levels at 30 min intervals for a further 7 hr (total 10 hr).

2.4 Biochemical measures

Plasma was prepared by centrifugation (GS-6R centrifuge; Beckman Coulter Inc, Brea, CA, USA) at 2095g for 10 min at 4°C, within 30 min of collection. Serum was left at room temperature for 30 min to allow for clot formation and then centrifuged at 2850g for 15 min at 4°C. The resulting plasma/serum was collected and stored at -80°C until analysed. Samples from each subject were analysed within the same analytic run to reduce variation.

Plasma glucose and serum total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and NEFA were measured using commercial enzymatic kits (Beckman Coulter Inc, Brea, CA, USA and Randox Laboratories Ltd, County Antrim, UK) on a Beckman AU480 clinical analyser (Beckman Coulter Inc, Brea, CA, USA). Plasma insulin, GIP, active GLP-1, ghrelin and PYY were measured using a magnetic bead Milliplex Human Metabolic Hormone Assay kit (Millipore), with intra- and inter-assay variability < 10 and <15% respectively. Plasma nitrotyrosine was measured by ELISA (Immun Diagnostik) with intra- and inter-assay variability of 6.2 and 7.9%. Plasma sICAM1 was measured by a Human sICAM-1 Platnium ELISA (EBioscience) with intra- and inter-assay variability of 4.1 and 7.7%.

2.5 Satiety and craving measurement

Satiety was measured via a four-item survey including general hunger, satisfaction, fullness and desire to eat scales. Each question was answered via a 10-point likert response scale with higher scores indicating higher satiety levels (i.e., decreased hunger). Total satiety was calculated as the mean response across all four items at each time point.

Cravings were measured via a four-item survey assessing desire to eat sweet, salty, savoury and fatty scales. Each question was answered via a 10-point likert response scale with

higher scores indicating higher cravings (i.e., increased hunger). Total cravings was calculated as the mean response across all four items at each time point.

2.6 Statistical analyses

The sample size calculation used previous data on glucose incremental areas under the curves (IAUC, mmol glucose min/L) from low fibre and high fibre foods with an average standard deviation of 35 mmol and correlation for repeated measures of 0.5, a sample size of 20 was calculated at 80% power (at $P<0.05$, two-tailed) to detect a change of 23 units. Withdrawal of up to 5 participants during the intervention has been allowed whilst maintaining the ability to detect an acceptable change in IAUC.

All statistical analyses were performed using SPSS (version 23.0), with $P \leq 0.05$ considered to be significant.

Participant characteristic data along with energy and nutrient intake data from visit 1 were averaged at baseline, data was presented as means ± sem.

The postprandial plasma glucose, hormones, I-CAM-1 and nitrotyrosine response for each test bread were compared by repeated-measures ANOVA. The IAUCs were calculated for these analytes using the trapezoidal rule and ignoring the area below the baseline. The IAUCs were compared by ANOVA using a 2x2 model (amylose x flour processing). The maximal concentration, time to reach maximal concentration and postprandial response curves were also calculated for plasma glucose and the data was assessed by ANOVA using a 2x2 model (amylose x flour processing). Breath hydrogen levels were reported as change from baseline (3 ½ hr following meal consumption) and the change from baseline was compared between each test bread by a repeated measures ANOVA and Tukey's pot-hoc test.

Change in satiety and hunger was assessed at 30, 60, 120 and 180 min post consumption. At each time point, change was assessed using Analysis of Covariance (ANCOVA). The relevant hunger or satiety scores were entered as the dependent variable with the baseline (0 min) entered as a covariate. Treatment (including Glucose) was entered as the independent factor, with Bonferroni post-hoc comparisons used to explore main treatment effects when present. A second set of 2x2 models (amylose x flour processing) were also conducted using ANCOVAs.

3 Results

3.1 Baseline characteristics of study participants

Participants had fasted blood cholesterol, lipids and glucose in the healthy range (Table 1). The macronutrient composition of the participants' habitual diet was 25% protein, 22% fat and 53% carbohydrate. The habitual diet was low in fibre (24 g/d) and only 27% of the grain-based foods consumed were wholegrain, which equated to an average of 1.7 wholegrain serves per day (Table 1).

3.2 Amylose level of bread altered glycemic, insulinemic and incretin response

During the 3 hr test period, the HAW breads had a 39% lower glycemic response (Figure 2) and 24% lower insulinemic response (Figure 3) compared to the LAW breads. The reduction in glycemic response for the HAW breads was most evident at 30 min at which the HAW breads had a lower rise in plasma glucose level compared with the wholemeal LAW bread (Figure 2). The insulinemic response was similar for all breads at 15 and 30 min.

At each time point during the 3 hr test period, the plasma GIP and GLP-1 concentrations were similar for all test breads (Figure 4 and 5). However, the HAW breads had a 30% lower GIP and GLP-1 AUC compared to the LAW breads (Figure 4 and 5).

3.3 Refinement of flour did not influence glycemic, insulinemic and incretin response in breads

The wholemeal and refined flour breads showed similar glycemic and insulinemic response (Figure 2 and 3). The incretin response (GIP and GLP-1) was also similar for the wholemeal and refined flour breads (Figure 4 and 5).

3.4 Amylose content did not affect satiety

Measures of subjective satiety were different between the treatment groups at 30 min [$F(4,79)=6.73, p<.001$], 60 min [$F(4,82)=3.81, p<.01$], 120 min [$F(4,83)=7.18, p<.001$] and 180 min [$F(4,83)=4.63, p<.01$] post consumption (Figure 6). At 30 min, satiety was highest following consumption of all test breads compared to the glucose drink (all $p<.05$). At 60 min, satiety levels remained higher for LAW wholemeal bread compared to the glucose drink ($p<.01$), but was similar for the other test breads. At 120 and 180 min, satiety levels were higher than glucose for the wholemeal breads (LAW and HAW) and refined wheat breads made from HAW flour. However, during the postprandial period satiety was similar for the four test breads ($P>0.05$). In addition, flour processing and amylose content had no effect on satiety.

Subjective craving measures at 30 min [F(4,79)=3.34,p<.05] and 60 min [F(4,82)=2.67,p<.05] were different between groups (Figure 7). At 30 min, craving levels were lower only for the LAW and HAW wholegrain breads compared to the glucose drink (all p<.05). At 60 min, only the LAW wholegrain bread had lower craving levels than the glucose drink (p<.05). However, during the postprandial period cravings were similar for the four test breads (all p>.05). Additionally, flour processing and amylose content had no effect on craving levels (all p>.05).

Plasma ghrelin levels decreased following test bread consumption, reaching nadir after 60 min, before returning to levels above baseline by 180 min (Figure 5). The amylose content and flour processing (wholemeal or refined) did not differentially affect the ghrelin response of the test breads (Figure 8).

3.5 Intestinal fermentation

Breath hydrogen levels started to increase 3 ½ h after the test breads were consumed (Figure 9). They continued to rise for the duration of the testing period (10 hr) however there was no differential effect of amylose content or flour processing (wholemeal or refined) on breath hydrogen levels.

Plasma PYY, a hormone secreted from enteroendocrine cells in response to short chain fatty acids production in the large intestine, did not change during the 3 hr postprandial period (Figure 10). Differences in the amylose content and processing (wholemeal or refined) of the flour used to make the test breads had no effect on plasma PYY levels during the 3 hr postprandial period (Figure 10).

3.6 Amylose content and flour processing did not affect markers of oxidative stress and inflammation

I-CAM-1 and nitrotyrosine levels showed a small decrease 60 min after the test breads were consumed, but by 180 min levels had returned to baseline or increased above baseline (Figure 11). Plasma I-CAM-1 and nitrotyrosine levels were not differentially affected by the amylose content of the bread or flour processing (wholemeal or refined) (Figure 11).

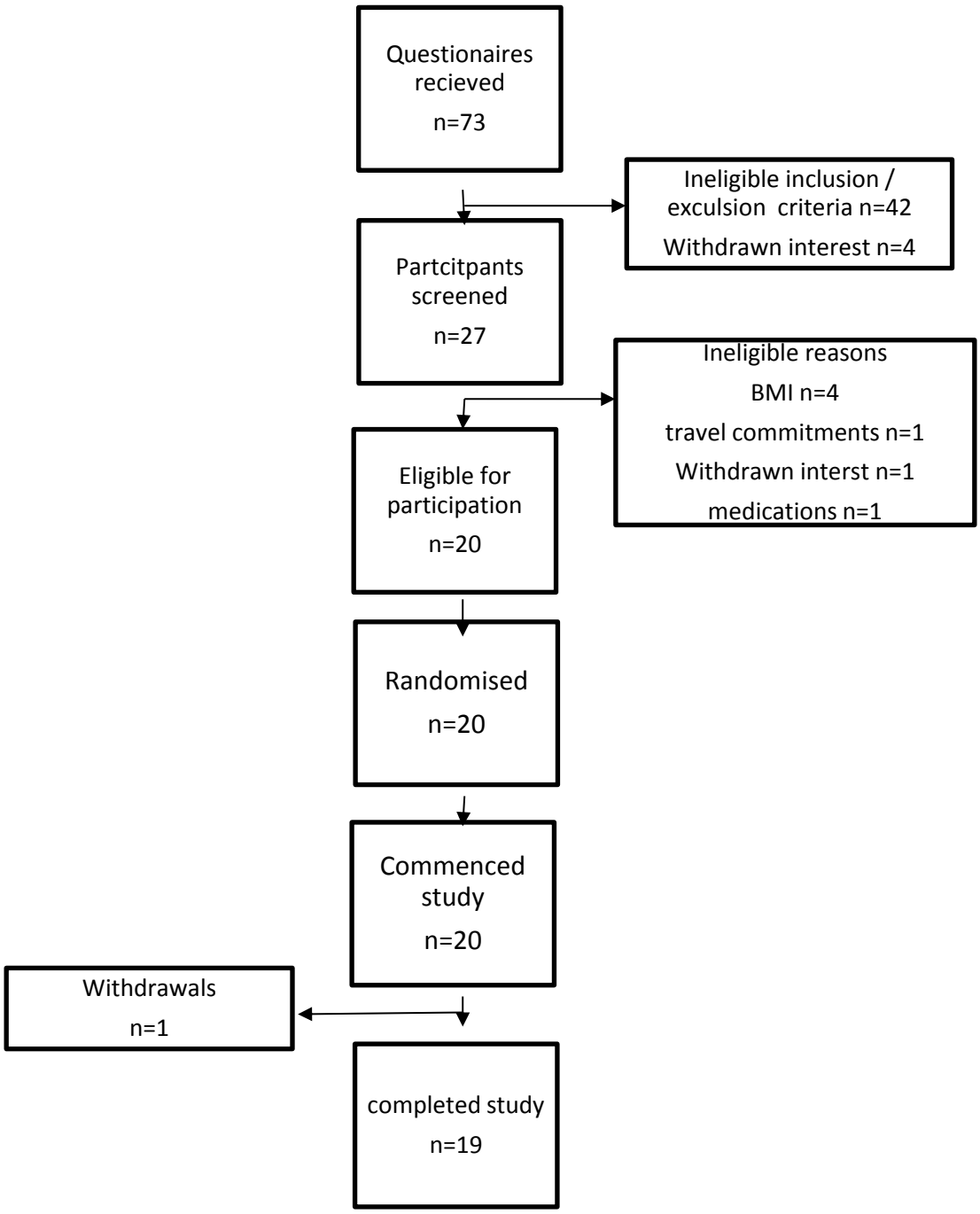


Figure 1 Participant flow diagram

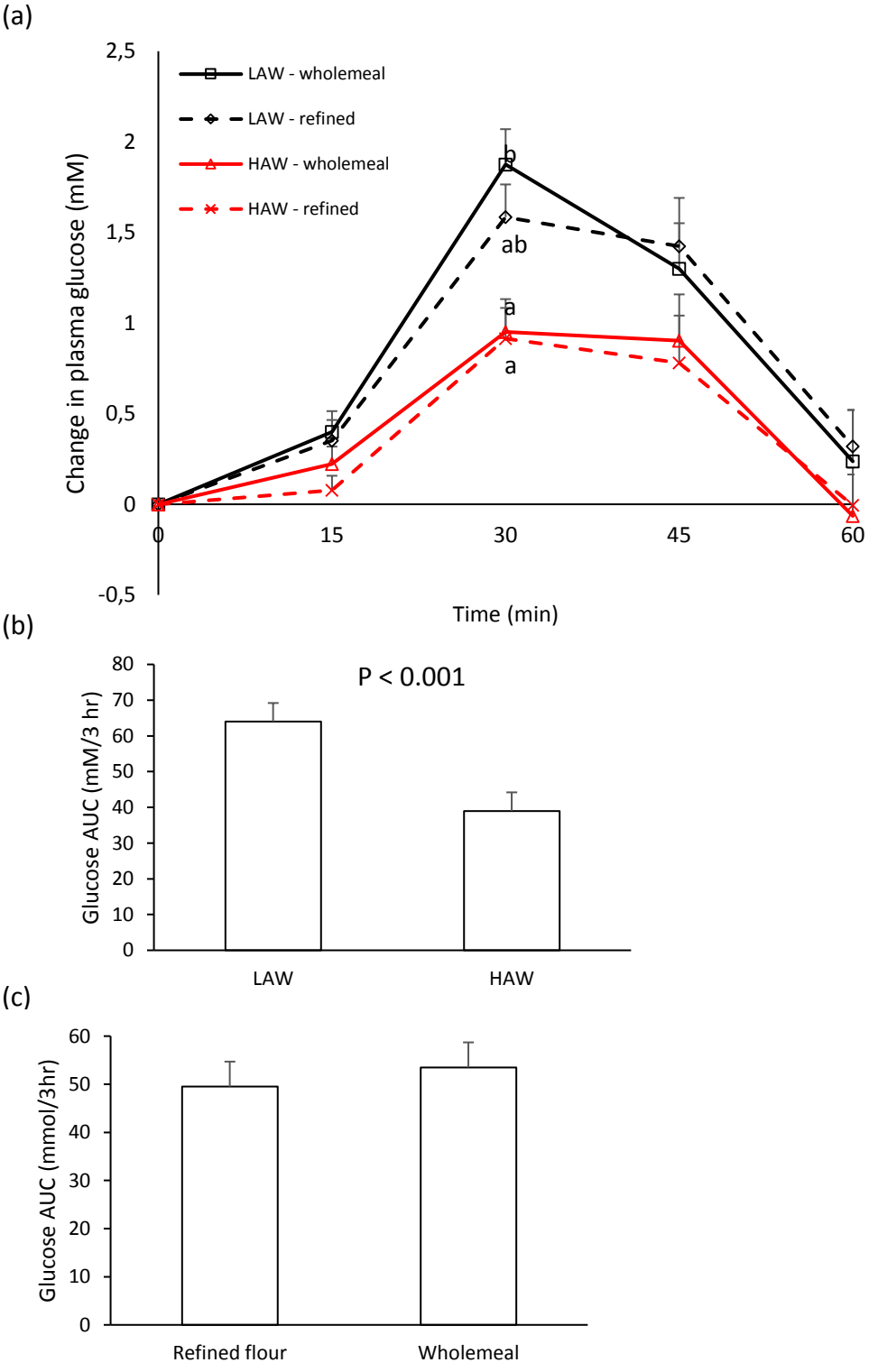


Figure 2 Mean ± SE glycemic response after consumption of breads made from LAW or HAW flour.
(a) Serum postprandial glucose concentration and incremental glucose AUC during the 3 hr test period are shown for breads that differ in (b) amylose content and (c) flour processing. At each time point, a different lowercase letter indicated means were significantly different from each other ($P \leq 0.05$). Sample size was 19.

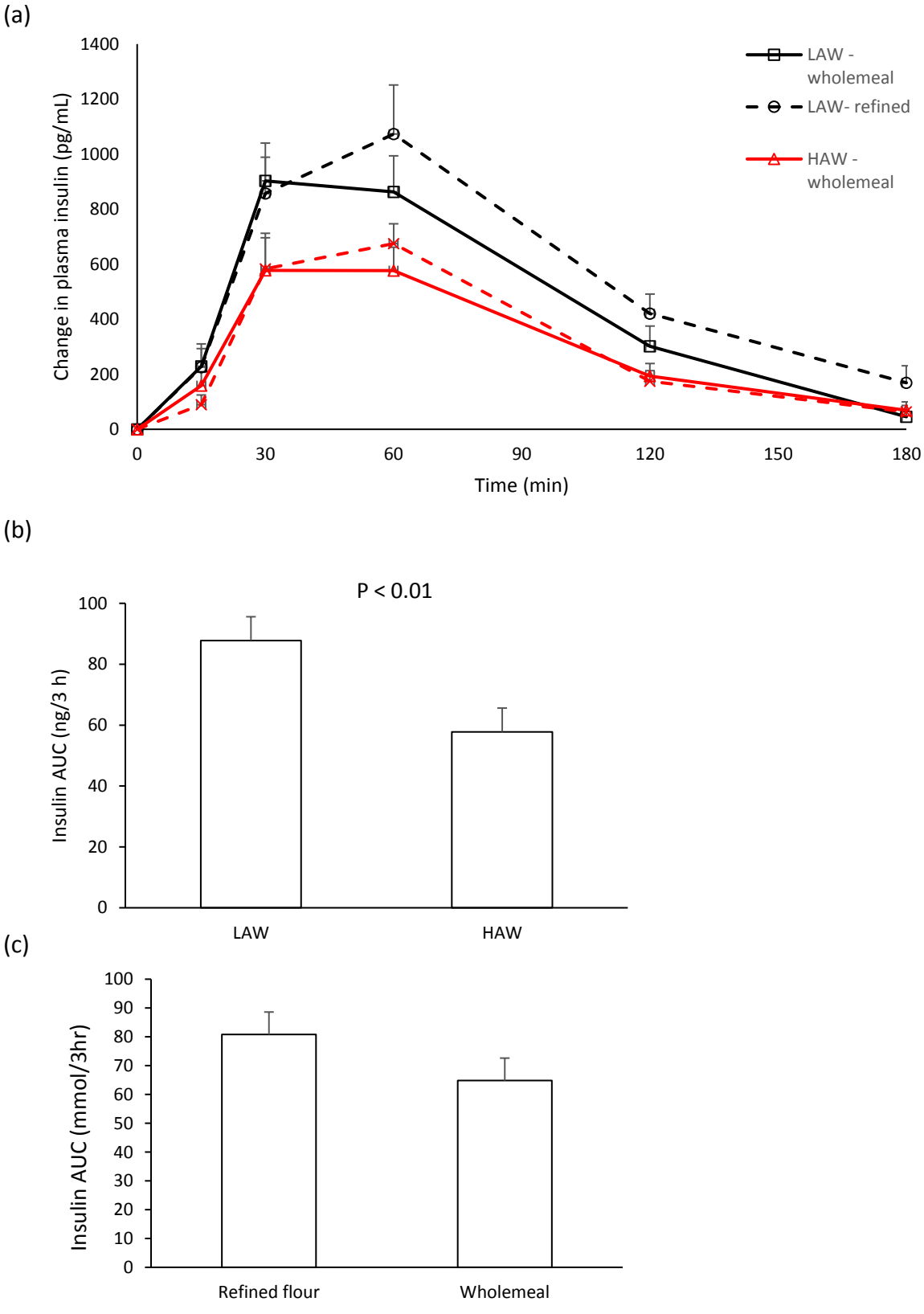
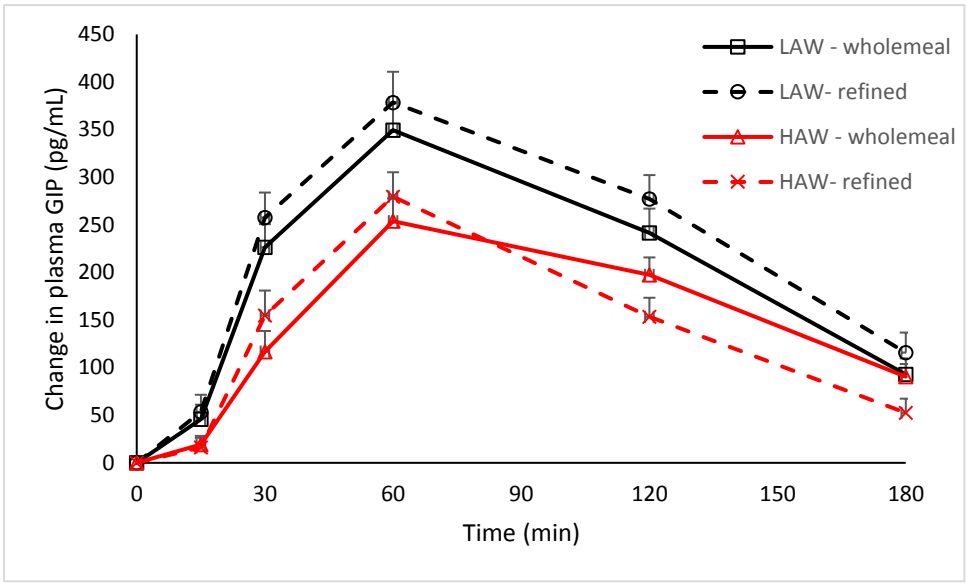
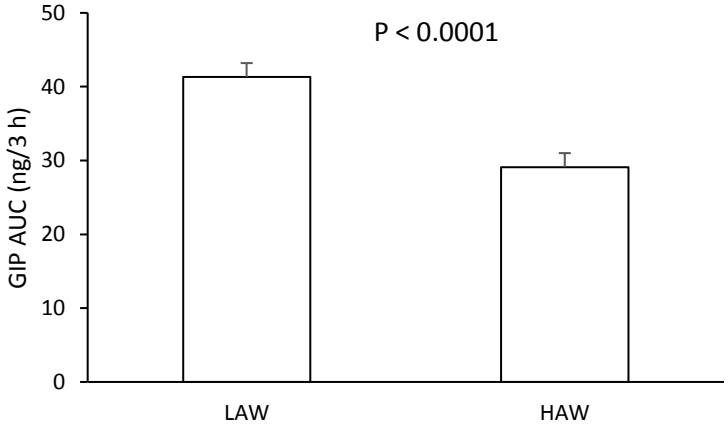


Figure 3 Mean ± SE insulinemic response after the consumption of breads made from LAW or HAW flour. (a) Serum postprandial glucose concentration and incremental glucose AUC during the 3 hr test period are shown for breads that differ in (b) amylose content and (c) flour processing. At each time point, a different lowercase letter indicated means were significantly different from each other ($P \leq 0.05$). Sample size was 19.

(a)



(b)



(c)

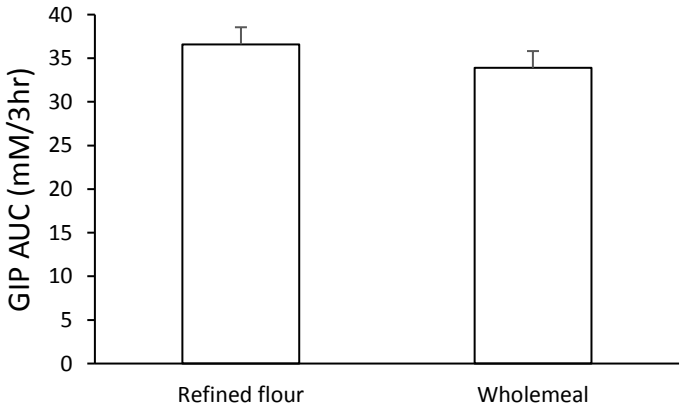


Figure 4 Mean ± SE gastric inhibitory peptide (GIP) response after the consumption of breads made from LAW or HAW flour.

(a) Serum postprandial glucose concentration and incremental glucose AUC during the 3 hr test period are shown for breads that differ in (b) amylose content and (c) flour processing. At each time point, a different lowercase letter indicated means were significantly different from each other ($P \leq 0.05$). Sample size was 19.

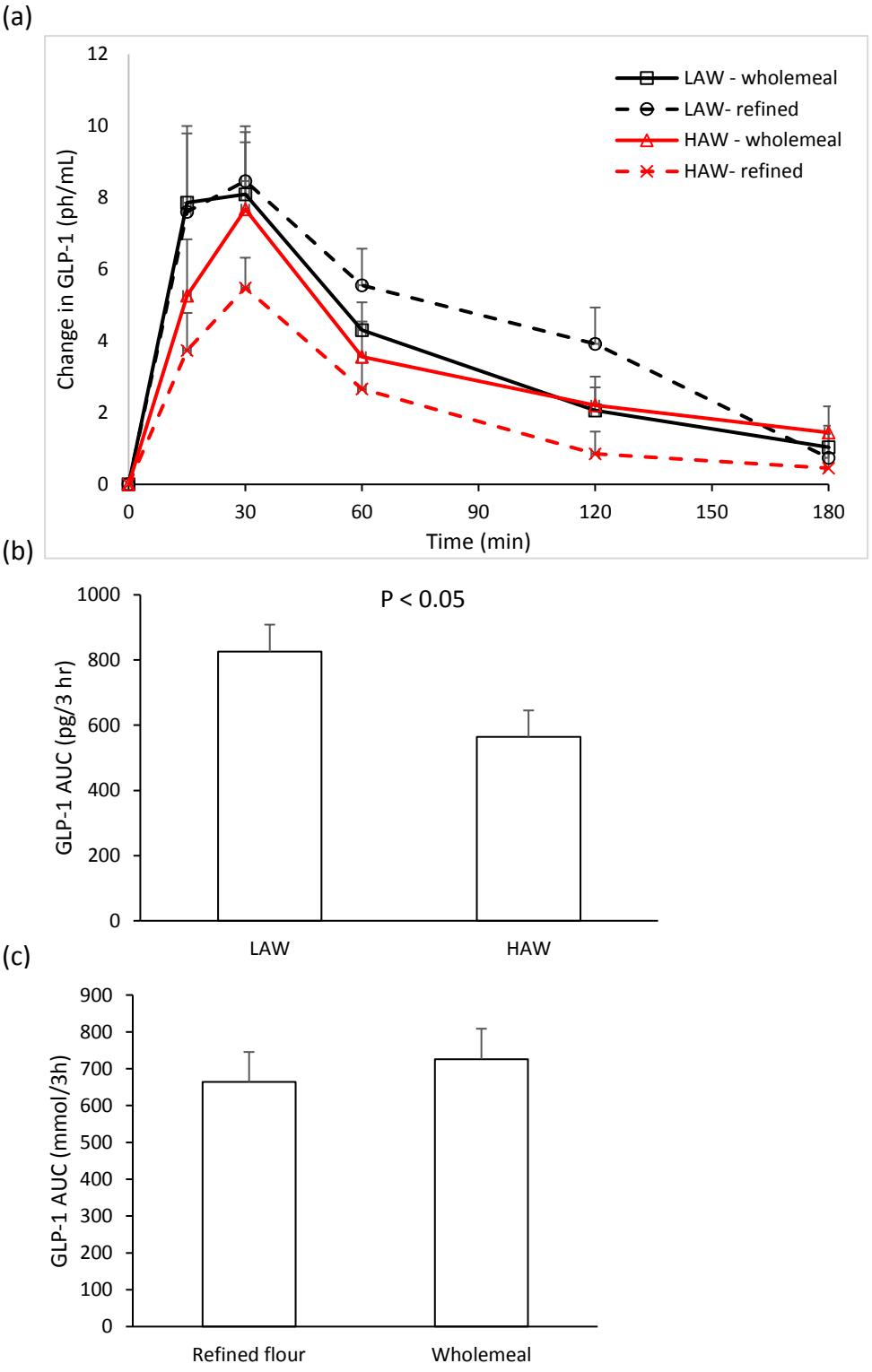


Figure 5 Mean ± SE glucagon-like peptide-1 (GLP-1) response after the consumption of breads made from LAW or HAW flour.

(a) Serum postprandial glucose concentration and incremental glucose AUC during the 3 hr test period are shown for breads that differ in (b) amylose content and (c) flour processing. At each time point, a different lowercase letter indicated means were significantly different from each other ($P \leq 0.05$). Sample size was 19.

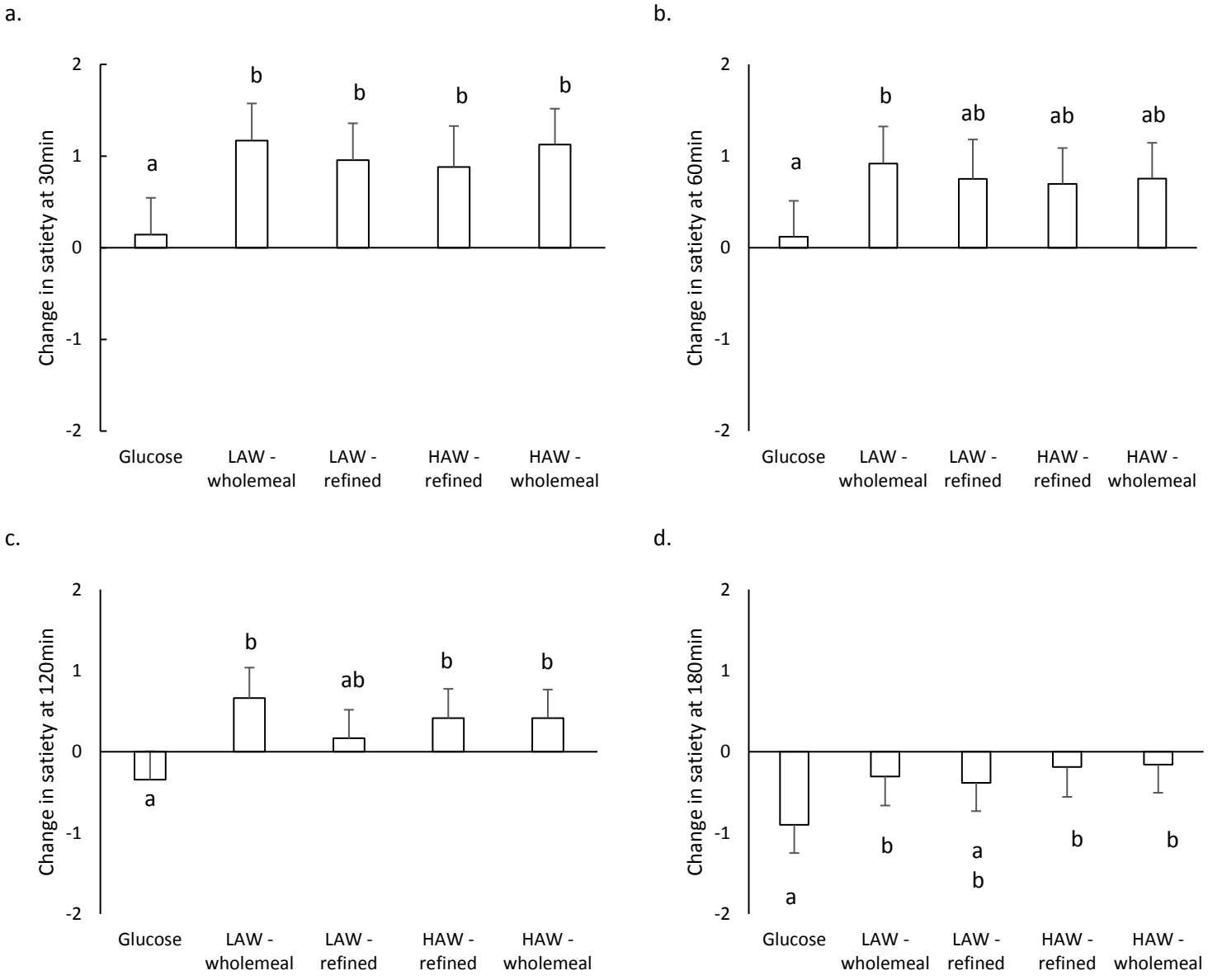
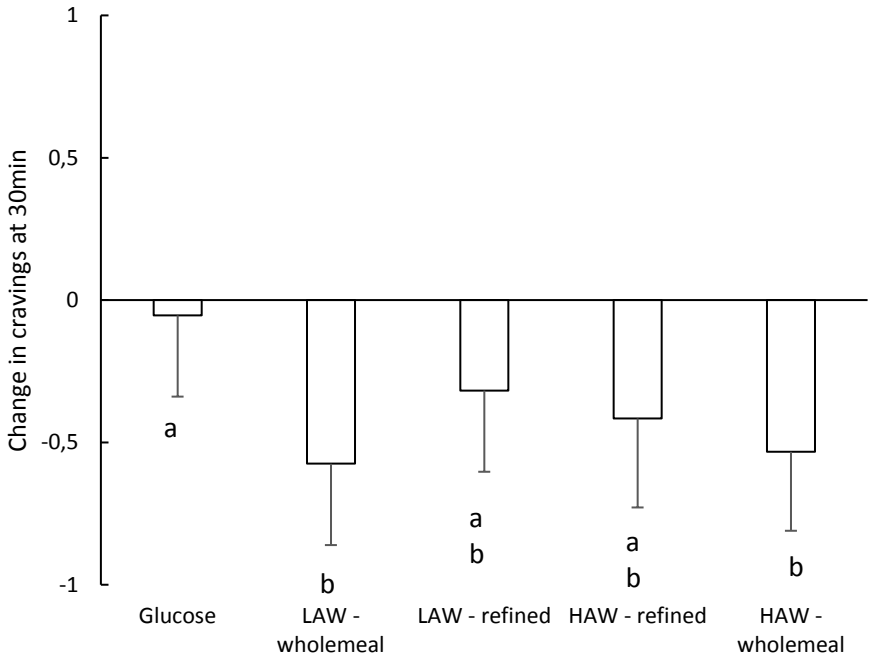


Figure 6 Changes in satiety at (a) 30, (b) 60, (c) 120 and (d) 180 min after the consumption of breads made from LAW or HAW flour.

The data is presented as degree of change in standard deviation units relative to baseline. Error bars represent 2.5*SE. A different letter denotes significant difference (P<0.05). Total sample size was 19.

a.



b.

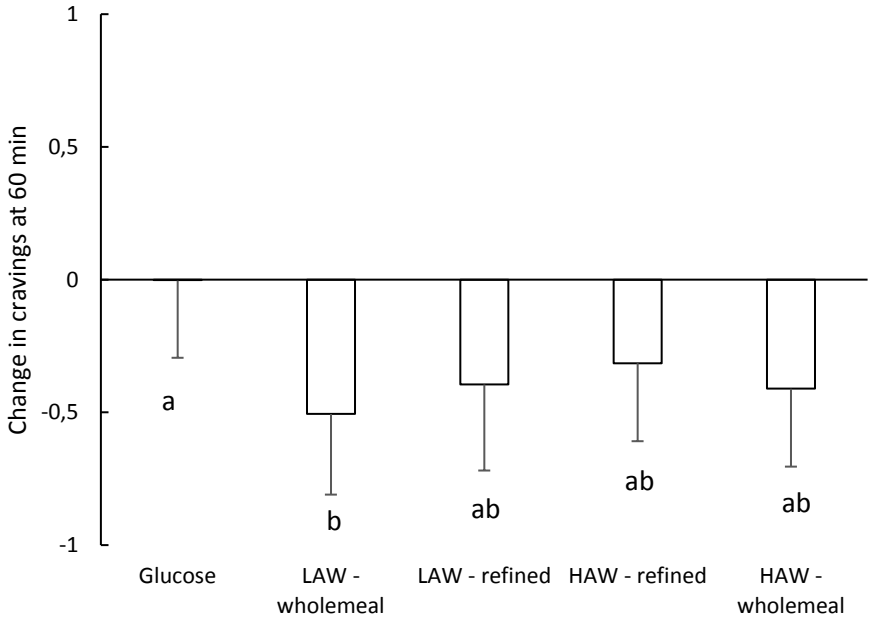


Figure 7 Changes in cravings at 30 and 60 min after the consumption of breads made from LAW or HAW flour.

The data is presented as degree of change in standard deviation units relative to baseline. Error bars represent 2.5*SE. A different letter denotes significant difference (P<0.05). Total sample size was 19.

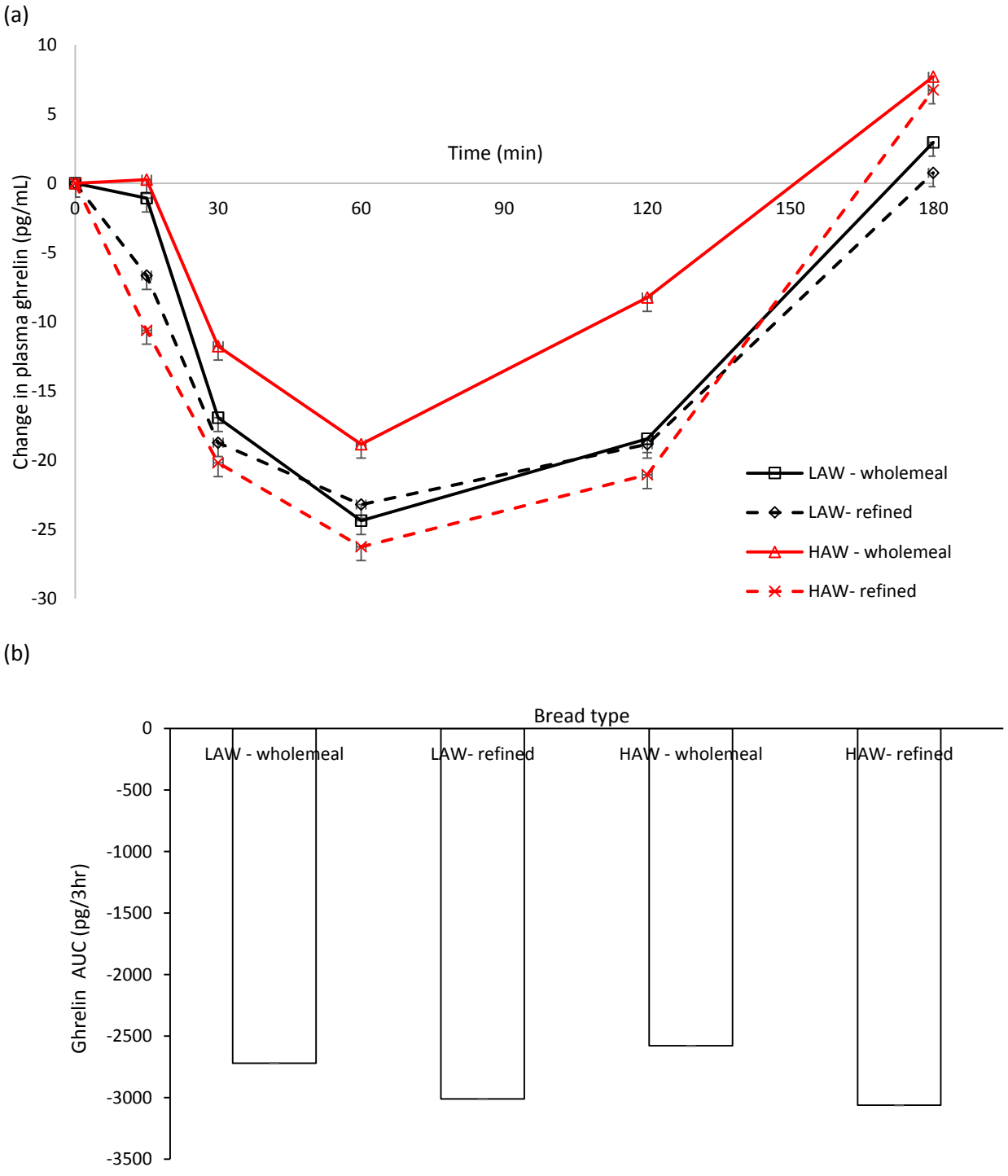


Figure 8 Mean ± SE ghrelin responses (a) and area-under-the-curve (b) after the consumption of breads made from low amylose wheat (LAW) or high amylose wheat (HAW) flour.

Differences in the response over time were determined with the use of a repeated measures ANOVA to assess the main effects; bread type, time and the bread type x time (not significant at $P \leq 0.05$). Total sample size was 19.

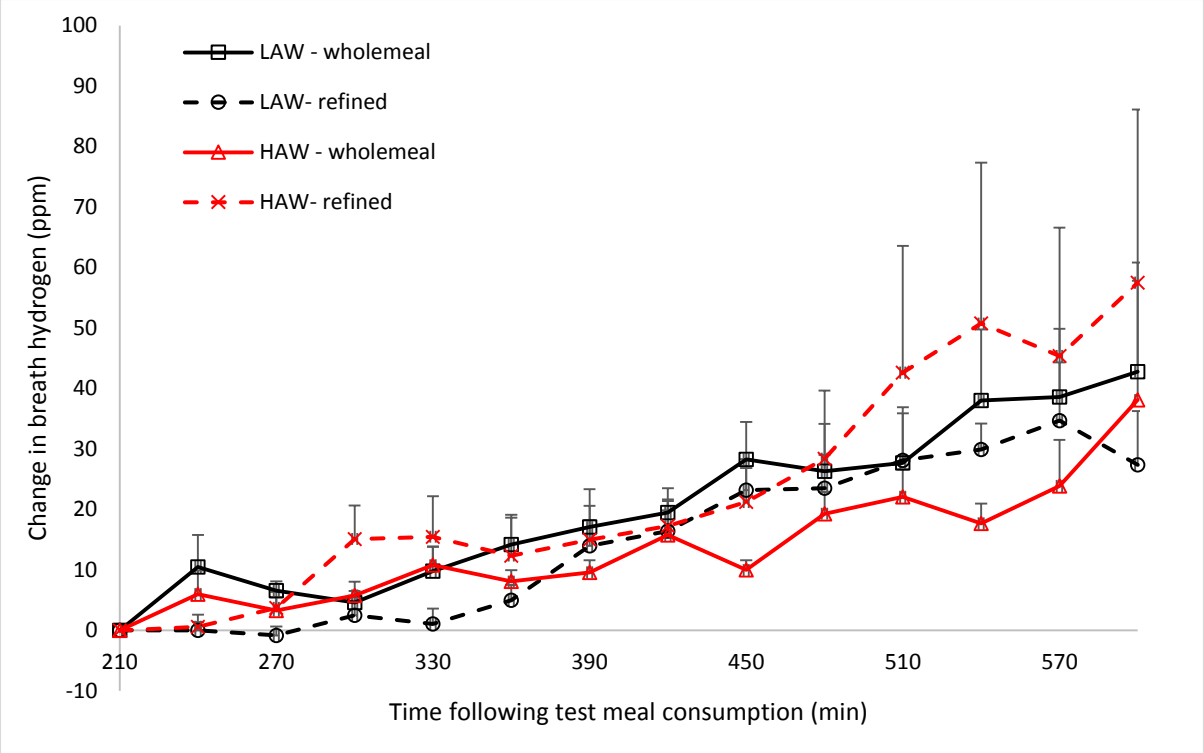


Figure 9 Mean ± SE breath hydrogen response after the consumption of breads made from LAW or HAW flour.

Differences in the response over time were determined with the use of a repeated measures ANOVA to assess the main effects; bread type, time and the bread type x time (not significant at $P \leq 0.05$). Total sample size was 4 as data from 2 individuals were excluded as their breath hydrogen levels did not increase over baseline levels.

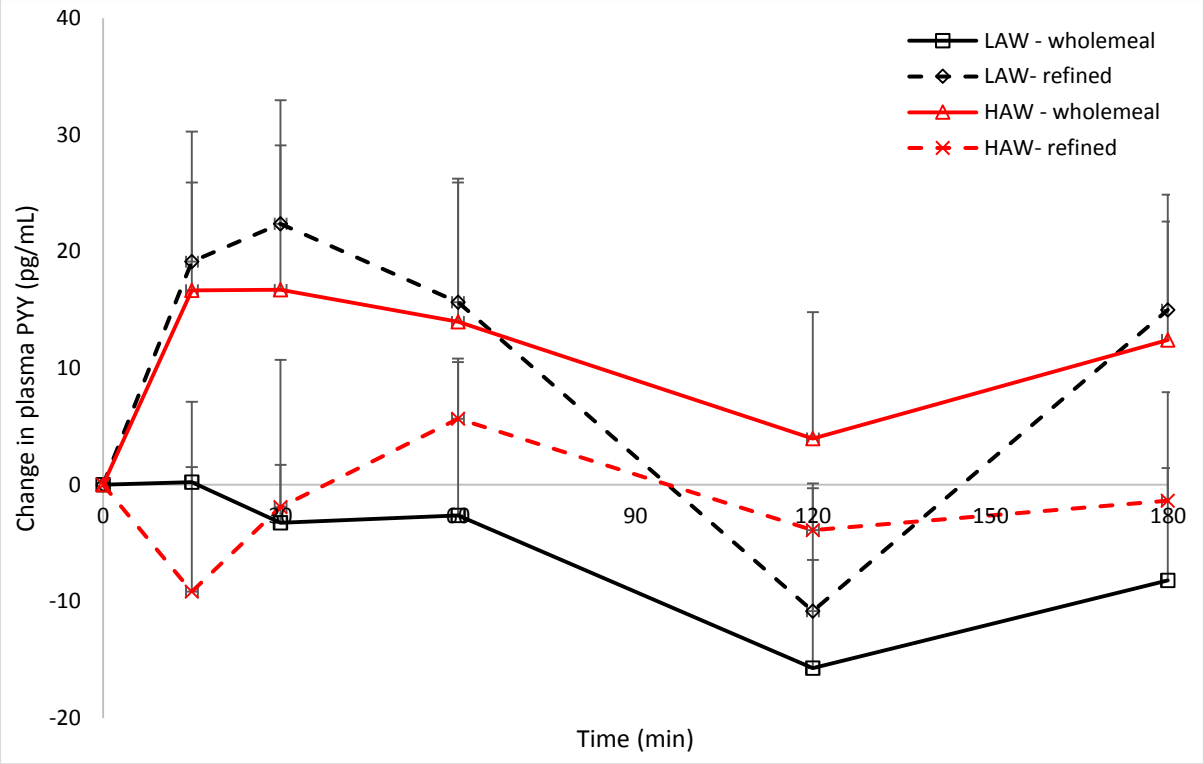


Figure 10 Mean ± SE peptide-YY (PYY) response after the consumption of breads made from LAW or HAW flour.

Differences in the response over time were determined with the use of a repeated measures ANOVA to assess the main effects; bread type, time and the bread type x time (not significant at $P \leq 0.05$). Total sample size was 19.

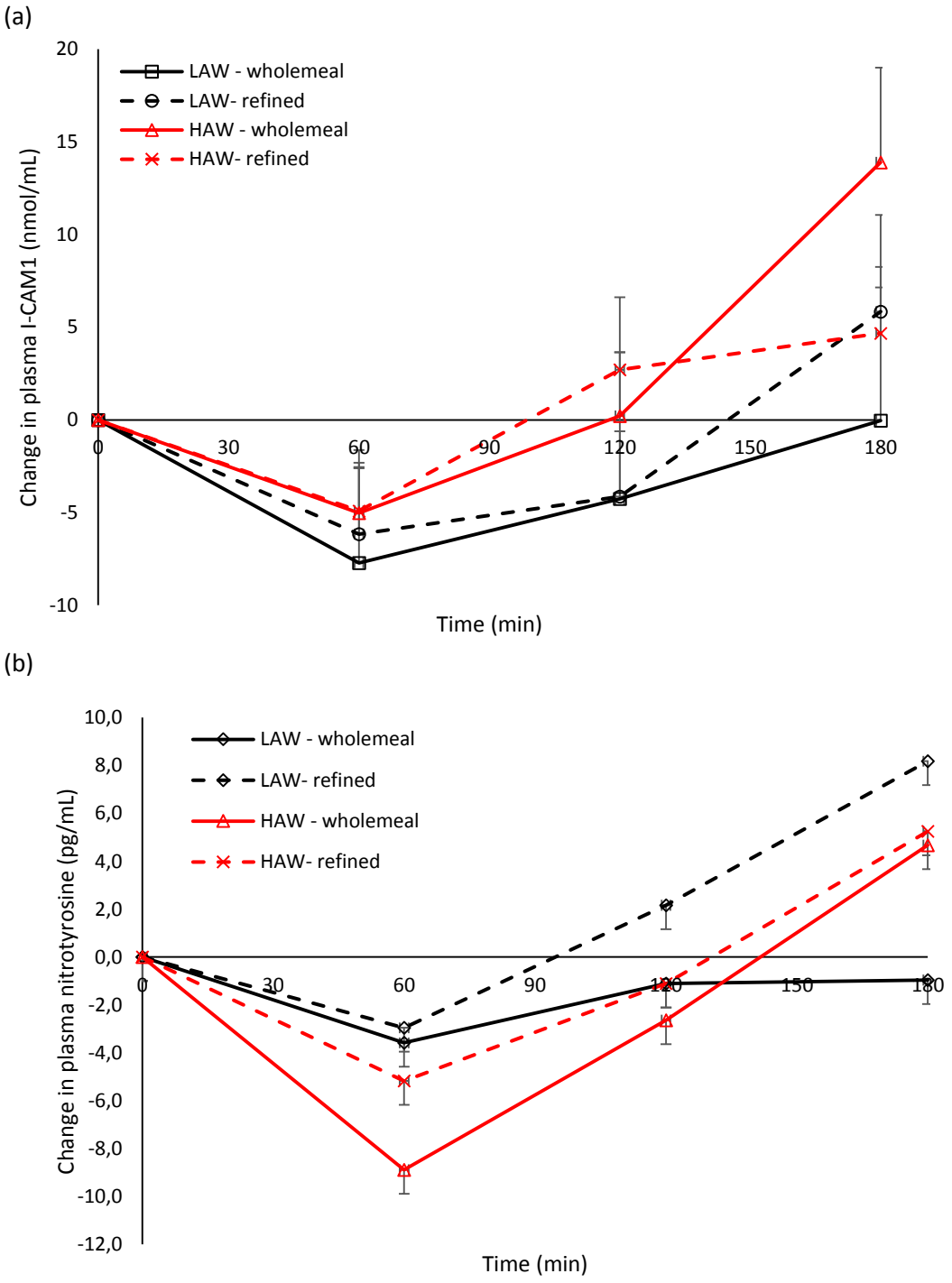


Figure 11 Mean ± SE (a) I-CAM-1 and (b) Nitrotyrosine responses after the consumption of breads made from LAW or HAW flour.

Differences in the response over time were determined with the use of a repeated measures ANOVA to assess the main effects; bread type, time and the bread type x time (not significant at $P \leq 0.05$). Total sample size was 19.

4 Discussion

Findings from the current study show that substitution of conventional wheat flour with HAW flour lowered the postprandial glycemic response of bread by 30%. This reduced glycemic response for HAW bread is consistent with a concomitant lowering of circulating levels of the incretin hormones, GIP and GLP-1 that are secreted in response to the presence of glucose in the duodenum and small intestine. Consistent with these changes in glycemia and incretin hormones during the postprandial period, the insulinemic response for HAW bread was 30% lower than LAW bread. HAW-induced dampening of postprandial glycemia could be due to a number of factors, including less available carbohydrate in these breads compared to control (conventional) breads. The compact structure of starch with elevated amylose content may also restrict starch swelling and gelatinisation, reducing the rate and extent of digestion (Akerberg et al. 1998, Panlasigui et al. 1991). Additionally, changes in the amylopectin structure in high amylose wheat could also contribute to reduced starch digestion rates.

The lower glycemic and insulinemic response of the HAW breads seen in the current study are consistent with a range of foods that have used other types of cereals that contain high levels of amylose (Al-Tamimi et al. 2010, Aldughpassi et al. 2012, Behall and Scholfield 2005, Ekstrom et al. 2013, Granfeldt et al. 1995, King et al. 2008, Klosterbuer et al. 2012). For instance, arepas made from high amylose corn starch (70% amylose) had a lower glycemic and insulinemic response than arepas made from conventional corn starch (25% amylose). A similar lowering of glycemia and insulinemia in men and women was also observed when high amylose maize starch was added to wheat-based breads. Importantly, amylose had to comprise at least 50% of total starch for this functional effect to occur (Behall and Hallfrisch 2002). When bread was made from wholegrain wheat containing only slightly elevated levels of amylose (38%) the glycemic response was essentially no different to that of bread made from conventional wholegrain wheat (Hallstrom et al. 2011).

If incorporated into the regular diet, substitution of conventional wheat with HAW could contribute to the prevention and/or management of type-2 diabetes. In relation to glucose sensitivity, a study showed a reduced glucose AUC in adults with prediabetes or type-2 diabetes after consumption of rice or bagels that contained higher levels of RS (Kwak et al. 2012). However, other studies have shown that glucose sensitivity is not improved following RS food interventions conducted in healthy (Behall and Howe 1995, Behall et al. 1989, Robertson et al. 2005, Weickert et al. 2005), overweight (Noakes et al. 1996) or hyperinsulinemic adults (Behall and Howe 1995). There are a number of factors that may explain the variable findings, including food form, type of processing, amount of RS and study duration. Long term consumption of foods high in RS may also improve insulin sensitivity by lowering the amount of insulin needed to stimulate glucose uptake. A study by Dainty et al (Dainty et al. 2016) showed that daily consumption of a bagel containing 25 g of high amylose maize starch improved glycemic disposal by reducing the amount of insulin required to manage postprandial glucose while improving fasting insulin sensitivity in adults at risk of diabetes. An earlier study by Behall et al (Behall et al. 1989) also showed that consumption of foods containing high amylose corn starch for five weeks reduced glycemic and insulinemic postprandial responses.

In the current study, processing of flour had little effect on glycemic response, even though the wholemeal breads had more than double the fibre content and up to 30% less starch than breads made from refined flour. This is consistent with previous studies which have shown that white and wholemeal breads have similar postprandial glycaemic responses in both diabetic volunteers and healthy adults (Jenkins et al. 1986, Kristensen et al. 2010, Scazzina et al. 2009). In addition, dietary intervention studies showed that conventional markers of glycemic control were similar when whole-grain or refined-grain products (predominantly bread made with milled whole wheat) were consumed by volunteers for 6 to 12 weeks (Andersson et al. 2007, Jenkins et al. 2002). Unlike other cereals such as oat and barley, the fibre content of wheat is predominately insoluble fibre which has limited functionality in modulating the glycemic impact of bread and other strategies are required to modulate starch digestion and/or absorption (Scazzina et al. 2013). In the current study, the similar glycaemic and insulinemic response for the HAW breads made from refined and wholemeal flours is likely due to complementary differences in the total amount of starch and resistant starch; compared to refined HAW bread, wholemeal HAW bread had less starch (refined 28.5 g/100g, wholemeal 19.9 g/100g) and less of this starch was resistant starch (refined 4.7 g/100g, wholemeal 3.2 g/100g). Importantly, this study demonstrates that substitution of conventional wheat flours with either refined or wholemeal wheat can be an effective approach to lower the glycemic response of bread.

In the current study we observed that certain test breads elicited greater satiety and reduced cravings at some time points during the postprandial period compared to the glucose control. However, throughout this period, satiety and cravings were similar between the four breads, which suggests that the amylose content of the breads and flour processing had no effect on these endpoints. This finding is consistent with a previous acute study that evaluated breakfast meals containing resistant starch and pullulan that lowered postprandial glucose, insulin, and GLP-1 response, but had no effect on satiety (Klosterbuer et al. 2012). Another study showed improvements in subjective appetite ratings, but this only occurred when white wheat bread was used as a control and medium weight guar gum was combined with a high amylose whole grain corn flour (Ekstrom et al. 2013). Importantly, the HAW breads used in the current study provided less energy (9 - 12% less kJ/100g) than the LAW control bread, which suggests that ongoing consumption could contribute to reducing total daily energy intake and the subsequent weight gain.

RS may provide the additional benefits of improving bowel health as short-chain fatty acids (SCFA) are major end products of the fermentation of NSP and RS by the microflora (Topping and Clifton 2001), and they promote important aspects of large bowel function. RS intakes are low in populations at high risk of the diseases of affluence, and there is a case for increasing RS consumption as an effective means of improving nutrition for public health at the population level. The current study had limited power to determine the effects of amylose content and flour processing on intestinal fermentation as only a small subset of individuals provided breath hydrogen samples. Furthermore, as bloods were only collected during the 3 hr postprandial period the RS in the test meals had not commenced fermentation in the large intestine, thus circulating peptides such as PYY remained at background levels. Subsequently, a clinical trial designed to thoroughly assess the prolonged effects of consuming high amylose wheat on a range of measures of fermentation and gut health is warranted. Markers of inflammation and oxidative stress also remained unchanged during the postprandial period which was surprising given that a comparable study showed significant changes in these markers (Dickinson et al. 2008). Further examination of these and other related markers of inflammation and oxidative stress are warranted in studies of

longer duration given the high levels of phytochemicals in wholegrains which may have enhanced bioavailability following intestinal fermentation (Belobrajdic and Bird 2013).

Although the long term effects of resistant starch have been consistently demonstrated for improving glucose control, this could not be evaluated as this study was only designed to measure the acute effects of a food made from HAW. Furthermore, bread was the only test food used in this study, therefore it is not certain whether substituting conventional flour with HAW in other food products would result in a similar magnitude of lowering of glycemic and insulinemic response.

5 Summary

• This randomised, double-blinded, crossover controlled study determined whether breads made from High Amylose Wheat (HAW) dampen postprandial glycemia compared to conventional wheat (Low Amylose Wheat, LAW).

• On separate mornings, 20 healthy non-diabetic men and women consumed four different wholemeal or refined wheat breads made from conventional or high amylose wheat flour (serving size of 121 g).

• High amylose wheat breads had lower glycemic, insulinemic and incretin (GIP and GLP-1) responses than conventional wheat breads ($P<0.05$).

• Processing of the flour (wholemeal or refined) did not affect glycemic or other metabolic endpoints measured.

• Flour processing and amylose content of the breads did not affect plasma ghrelin or subjective measures of satiety or cravings during the postprandial period.

• Breath hydrogen levels started to increase 3 ½ hr after the test breads were consumed and continued to rise for the duration of the testing period (10 hr) however there was no differential effects of amylose content or flour processing (wholemeal or refined) on breath hydrogen levels.

• Amylose content and flour processing did not affect markers of oxidative stress (nitrotyrosine) or inflammation (I-CAM-1).

• These findings demonstrate that replacing conventional with high amylose wheat flour is an effective strategy for lowering postprandial glycemic and insulinemic responses to bread.

• Follow up clinical studies that examine prolonged consumption of foods containing HAW on improving glucose control in healthy individuals and adults with pre-diabetes is warranted.

• Further benefit from using high amylose flour in other wheat based food products such as ready-to-eat breakfast cereals and bars, noodles and doughs may result in the lowering of the glycemic impact of a range of different foods and deserves further investigation.

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