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High-Amylose Wheat Lowers the Postprandial Glycemic Response to Bread in Healthy Adults: A Randomized Controlled Crossover Trial

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Abstract

Background: Conventional wheat-based foods contain high concentrations of readily digestible starch that commonly give these foods a high postprandial glycemic response and may contribute to the development of type 2 diabetes and cardiovascular disease.

Objectives: The aim of this study was to determine if bread made from high-amylose wheat (HAW) and enriched in resistant starch dampens postprandial glycemia compared with bread made from conventional low-amylose wheat (LAW).

Methods: This single-center, randomized, double-blinded, crossover controlled study involved 7 consecutive weekly visits. On separate mornings, 20 healthy nondiabetic men and women (mean age 30 ± 3 y; body mass index 23 ± 0.7 kg/m^2) consumed a glucose beverage or 4 different breads (each 121 g); LAW-R (refined), LAW-W (wholemeal), HAW-R, or HAW-W. The starch contents of the LAW and HAW breads were 24% and 74% amylose, respectively. Venous blood samples were collected at regular intervals before and for 3 h after the breakfast meal to measure plasma glucose, insulin, ghrelin, and incretin hormone concentrations, and the incremental area under the curve (AUC) was calculated (mmol/L × 3 h). Satiety and cravings were also measured at 30-min intervals during the postprandial period.

Results: HAW breads had a glycemic response (AUC) that was 39% less than that achieved with conventional wheat breads (HAW 39 ± 5 mmol/L × 3 h; LAW 64 ± 5 mmol/L × 3 h; $P < 0.0001$). Insulinemic and incretin responses were 24–30% less for HAW breads than for LAW breads ($P < 0.05$). Processing of the flour (wholemeal or refined) did not affect the glycemic, insulinemic, or incretin response. The HAW breads did not influence plasma ghrelin, or subjective measures of satiety or cravings during the postprandial period.

Conclusions: Replacing LAW with HAW flour may be an effective strategy for lowering postprandial glycemic and insulinemic responses to bread in healthy men and women, but further research is warranted. This trial was registered at the Australian and New Zealand Clinical Trials Registry as ACTRN12616001289404. *J Nutr* 2019;00:1–11.

Keywords: wheat, amylose, resistant starch, fiber, glycemic, insulinemic, incretin, whole grain, bread, human

Introduction

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 wholegrain cereal foods is recognized as an important approach for reducing the risk of these prevalent health problems (1–4). The benefits of whole grains are largely attributed to their dietary fiber component, which includes nonstarch polysaccharides and resistant starch (RS).

Although people are eating more whole grains, refined starches from cereals, such as wheat, rice, and corn, that elicit a high glycemic response continue to be the main forms of carbohydrates consumed across the globe. To address this, corn and barley varieties, and food ingredients, have been developed that contain elevated RS concentrations by increasing the proportion of amylose in the starch. Amylose concentration and RS content are positively correlated. Foods made from high-amylose cereals induce a lower postprandial glycemic response in healthy individuals than foods made from conventional grains (5–15).

For processed cereal products, such as bread, pasta, and noodles, which supply ~20% of food calories for the world population, wheat is the preferred base flour. These foods typically contain low concentrations of RS. Conventional

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wheat starch typically contains 25–30% amylose, but can contain as much as 38% (16). However, bread made from wheat containing slightly higher amounts of amylose (38%) showed a similar glycemic and insulinemic response to that of conventional bread (17). A wheat variety containing more than double this concentration of amylose (85%) has been developed by our research group (18). Although the RS content of the grain has been shown to be increased 10-fold, the glycemic and metabolic properties are not yet known.

The primary aim of this study was to determine whether breads made from this high-amylose variety of wheat attenuated postprandial glycemia. Wholemeal and refined white flours were used to ascertain the influence of flour processing on glycemic response. To evaluate glucose handling more comprehensively, changes in circulating incretin hormones were also measured as well as breath hydrogen concentrations to determine upper gut intestinal transit rate. A 10-point Likert scale was used to assess feelings of fullness and cravings, along with a hormonal indicator of satiety (plasma ghrelin) because cereal-based foods containing RS have been shown to influence measures of satiety in some studies. To evaluate whether the acute glycemic response modulated measures of inflammation and oxidative stress, serum intercellular adhesion molecule 1 (ICAM-1) and nitrotyrosine concentrations were measured during the postprandial period.

Methods

Study population

Twenty volunteers (15 women, 5 men) with a mean age of 30 ± 3 y and BMI of 23 ± 0.7 kg/m^2 were included in the study. Inclusion criteria included: age 18–65 y, BMI 18.5–≤27.5, and a normal fasting blood glucose concentration of 3.5–5.5 mmol/L. The exclusion criteria included: known presence of diabetes, smoker, pregnant or lactating, sufferer of bleeding disorders, known food allergy, hypersensitivity or intolerance to wheat or starchy foods, taking medications known to influence glucose tolerance or gastric emptying (oral contraceptives 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 d 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 Research Ethics Committee. This study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12616001289404) and was conducted between October and December 2016.

Recruitment and screening

The participants were recruited from the CSIRO Nutrition and Health Research Clinic database and the CSIRO website from September 17, 2016 until November 4, 2016. To compensate participants for time spent attending the clinic, participants were provided with gift vouchers on completion of the study to an amount equivalent to time spent in the study (up to $245).

Participants were provided with information about the study design and, if interested, a first screening telephone questionnaire was administered to determine general eligibility. If eligible, at the first visit to the clinic, participants were acquainted with the study procedures and their fasting venous blood glucose concentration was checked to establish whether it was in the normal range (3.5–5.5 mmol/L). All eligible individuals were invited to commence the study.

Seventy-three volunteers were screened by telephone, and 20 participants were enrolled in the study. The flow of participants is depicted in **Figure 1**. Nineteen participants completed the study; 1 participant withdrew on the first visit of the study because of a needle phobia.

Study design and intervention

This work reports a single-center, randomized, double-blinded, crossover controlled study that involved 7 food challenges administered over 7 consecutive weekly visits. Three of the challenges consisted of an identical 300-mL glucose drink (Carbotest; Lomb Scientific) that contained 50 g glucose and were consumed only on visits 1, 4, and 7. These 3 glucose challenges were included for consistency with standard glycemic index testing and to evaluate the intraindividual variability of the glycemic response data (19). On visits 2, 3, 5, and 6 there were 4 bread challenges; high-amylose wheat refined (HAW-R), high-amylose wheat wholemeal (HAW-W), low-amylose wheat refined (LAW-R), and low-amylose wheat wholemeal (LAW-W). The order of test breads was randomly allocated to each individual through the use of a Latin square randomization sequence that included 4 unique sequences: ABDC, BCAD, CDBA, and DACB. Randomized allocation was conducted by the clinic manager who was also responsible for unblinding the data once statistical analysis had been completed by the project leader (DPB). The participants were instructed to maintain their dietary habits and daily routine for the duration of study (~8 wk), avoiding consuming foods high in fiber 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 also restricted on the day prior to testing.

Participants and clinic staff were blinded to the composition of each test bread, which was designated by differently colored labels. The test breads were only decoded once preliminary statistical analyses were completed. There were no discernible differences in taste, texture, or appearance for breads made from either HAW or conventional LAW flours. However, there were obvious differences between breads made from wholemeal and refined flour.

The 4 wholemeal or refined wheat breads were made from LAW or HAW flour according to a standard bread recipe. The amylose content of the LAW and HAW was 24% and 74.3%, respectively. The flour and other 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). After 15 min at ambient temperature, doughs were divided into 560-g portions, then shaped, placed into a pan, and proved 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 h 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 serving size of 121 g. Samples of each test bread were analyzed for starch, RS, sugar, total dietary fiber, fat, and protein content (**Table 1**). Freeze-dried and milled samples of each test bread were analyzed in duplicate according to standard Association of the Official Analytical Chemists methods. RS was measured by the method of McCleary and Monaghan (20). The 121-g servings of bread consumed by participants contained the following amounts of carbohydrate (sum of sugar and starch): LAW-R, 50 g; LAW-W and HAW-R, 40 g; HAW-W, 30 g. Additionally, starch digestibility varied markedly between the different bread products (Table 1).

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Supplemental Figure 1 is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/jn/.

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Abbreviations used: GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; HAW, high-amylose wheat; HAW-R, high-amylose wheat refined; HAW-W, high-amylose wheat wholemeal; IAUC, incremental area under the curve; ICAM-1, intercellular adhesion molecule 1; LAW, low-amylose wheat; LAW-R, low-amylose wheat refined; LAW-W, low-amylose wheat wholemeal; RS, resistant starch.

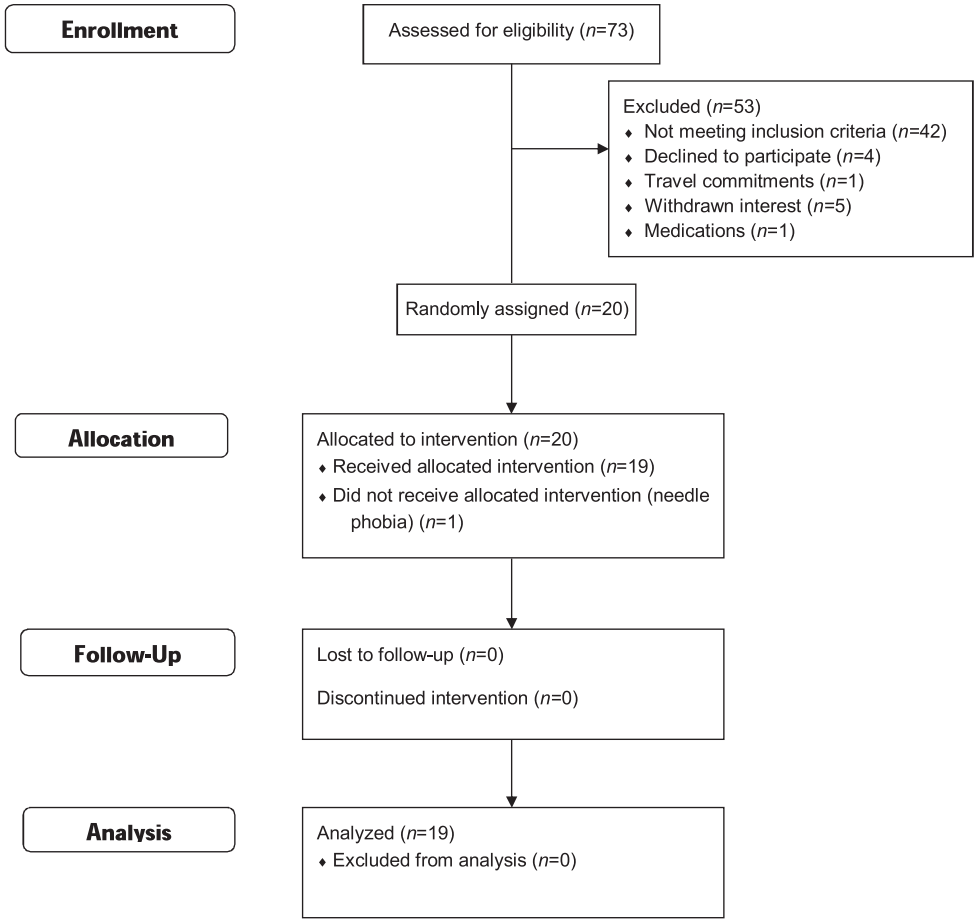

FIGURE 1 Flow diagram showing the recruitment process for the intervention study.

Participant baseline characteristics were obtained at the first visit to the clinic. These included height, which was measured with a stadiometer (SECA), and body weight, which was measured with calibrated electronic digital scales (AND HW-PW200); BMI was calculated as weight (in kg)/height (in m)^2. A 12-h fasting blood glucose concentration was determined in a finger prick blood sample by HemoQue. The mean value for participants was 4.6 ± 0.4 mmol/L and all participants had a value within the healthy range (3.5–5.5 mmol/L). Blood was also collected from the antecubital vein, and total cholesterol, LDL and HDL cholesterol, total triglyceride, and nonesterified free fatty acid concentrations were determined. During the week of the first visit, an assessment of the participant's typical diet and energy intakes was determined based on a 24-h food recall conducted by a dietitian via a telephone interview.

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

	LAW-R	LAW-W	HAW-R	HAW-W
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
Fiber	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
Energy	1,045	984	951	922

[1] Composition values are g/100 g; energy values are kJ/100 g. Energy content of bread determined based on energy values for protein (17 kJ/g), fat (37 kJ/g), carbohydrate (17 kJ/g), and total fiber (10 kJ/g). HAW, high-amylose wheat; HAW-R, high-amylose wheat refined; HAW-W, high-amylose wheat wholemeal; LAW, low-amylose wheat; LAW-R, low-amylose wheat refined; LAW-W, low-amylose wheat wholemeal.

On the morning of each meal test, participants arrived at the clinic following an overnight fast, and an intravenous cannula was inserted into 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 (the 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) for measurement of glucose; P800 tubes (BD Biosciences) for measurement of insulin, gastric inhibitory peptide (GIP), active glucagon-like peptide-1 (GLP-1), ghrelin, and peptide YY; and serum tubes (Vacuette, Greiner bio-one) for measurement of nitrotyrosine and ICAM-1. Blood samples collected in P800 and sodium fluoride tubes were immediately placed on ice and processed to obtain plasma within 15 min of collection. Perceived appetite, satiety, and mood were assessed with the use of a visual analog 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 concentrations could be determined (4 test breads only) with a Gastro+ Gastrolyser (Bedfont). 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 h, participants continued to measure their breath hydrogen concentrations at 30-min intervals for a further 7 h (total

10 h), which provided sufficient time to evaluate the commencement of intestinal fermentation and determine the upper-gut transit rate.

Biochemical measures

Plasma was prepared by centrifugation (GS-6R centrifuge; Beckman Coulter Inc.) at 2095 × *g* 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 2850 × *g* for 15 min at 4°C. The resulting plasma/serum was collected and stored at −80°C until analyzed. Samples from each subject were analyzed within the same analytic run to reduce variation.

Plasma glucose and serum total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, and nonesterified free fatty acids were measured with commercial enzymatic kits (Beckman Coulter Inc. and Randox Laboratories Ltd.) on a Beckman AU480 clinical analyzer (Beckman Coulter Inc.). Plasma insulin, GIP, active GLP-1, ghrelin, and peptide YY were measured with the use of a magnetic bead Milliplex Human Metabolic Hormone Assay kit (Millipore); the intra- and interassay variability was <10% and <15%, respectively. Plasma nitrotyrosine was measured by ELISA (Immun Diagnostik); the intra- and interassay variability was 6.2% and 7.9%, respectively. Plasma serum ICAM-1 was measured by Human sICAM-1 Platinum ELISA (EBioscience); the intra- and interassay variability was 4.1% and 7.7%, respectively.

Subjective satiety and cravings

Satiety and cravings were measured according to a validated subjective appetite sensations methodology (21). This approach is designed to measure the acute effects of meal consumption on these domains through the use of multiple repeated assessments [e.g., (22)]. Responses are elicited for satiety through the use of 4 items assessing general hunger, satisfaction, fullness, and desire to eat. Cravings are measured through the use of 4 items assessing the desire for sweet, salty, savory, and fatty foods. Each individual item was answered via a 10-point Likert response scale; higher scores indicating increased satiety (i.e., decreased hunger), or increased cravings. Scores across all 4 items within each domain (satiety or cravings) were averaged at each time point and converted to standardized scores, enabling the expression of the difference between baseline and later time points in standardized units to aid interpretation.

Upper-gut intestinal transit rate

Six of the 20 subjects were randomly selected to provide breath samples so that breath hydrogen concentrations could be determined for the 4 test breads through the use of a Gastro+ Gastrolyser (Bedfont). Breath hydrogen was measured at baseline and at 30-min intervals for 10 h following consumption of the test meal. Upper-gut intestinal transit time was determined by identifying the time at which breath hydrogen concentrations commence increasing (23).

Statistical analyses

The sample size calculation used previous data on the incremental area under the curve (IAUC) for glucose (mmol/L glucose × 2 h) from low-fiber and high-fiber foods with an average SD of 35 mmol/L glucose × 2 h and correlation for repeated measures of 0.5; a sample size of 20 was calculated at 80% power (at $P < 0.05$, 2-factor) to detect a change of 23 units. Withdrawal of up to 5 participants during the intervention maintained the ability to detect an acceptable change in IAUC.

All statistical analyses were performed with 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 are presented as means ± SEMs.

The postprandial plasma glucose, hormone, ICAM-1, and nitrotyrosine response for each test bread were compared by repeated-measures ANOVA. The mean glycemic response to a standardized glucose drink was calculated by averaging the 3 postprandial glucose responses for each individual. The IAUCs were calculated for these analytes according to the trapezoidal rule and ignoring the area below the baseline. For 4 participants, 1 of the breads tested (a different test bread for each individual) elicited an IAUC response that was >2 SD above the mean AUC, and was therefore excluded from analysis based on the standardized glycemic index testing protocol (19). The IAUCs were compared by ANOVA through the use of a 2 × 2 model (amylose × flour processing). The maximal concentration, time to reach maximal concentration, and postprandial response curves were also calculated for plasma glucose, and the data were assessed by ANOVA with the use of a 2 × 2 model (amylose × flour processing). Breath hydrogen concentrations were reported as change from baseline (3.5 h following meal consumption), and the change from baseline was compared between each test bread by a repeated-measures ANOVA and Tukey's post-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 through the use of ANCOVA. The relevant hunger or satiety score for that time point was 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 2 × 2 models (amylose × flour processing) was also conducted with the use of ANCOVAs following this same approach.

TABLE 2 Baseline participant characteristics[1]

Age, y	30 ± 2
Sex, *n* (%)	
F	14 (74%)
M	5 (26%)
Body weight and composition	
Body weight, kg	67 ± 3
BMI, kg/m^2	23 ± 0.6
Fasting whole blood glucose, mmol/L	4.6 ± 0.4
Cardiovascular disease risk markers, mmol/L	
Total serum cholesterol	4.8 ± 0.2
LDL serum cholesterol	0.9 ± 0.1
HDL serum cholesterol	1.5 ± 0.1
Serum triglycerides	2.8 ± 0.2
Plasma nonesterified fatty acids	0.4 ± 0.1
Typical diet (24-h recall)	
Total energy, kJ/d	8,046 ± 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 fiber, g/d	24 ± 2
Refined grain servings, numbers/d	4.5 ± 0.8
Wholegrain servings, numbers/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). Values are means ± SEMs, $n = 19$ (5 men/14 women).

Results

Baseline characteristics of study participants

Participants had fasting blood cholesterol, lipids, and glucose in the healthy range (**Table 2**). The macronutrient composition of the participants' typical diet was 25% protein, 22% fat, and 53% carbohydrate. The typical diet was adequate in fiber (24 g/d) and only 27% of the grain-based foods consumed were whole grain, which corresponds to 1.7 whole grain servings per day (Table 2).

TABLE 3 Plasma glucose and metabolic hormone response for healthy adults after consumption of breads that differ in amylose content and amount of flour processing[1]

	Amylose content			Flour processing		
	LAW	HAW	*P* value	Refined	Wholemeal	*P* value
Plasma glucose (mmol/L × 3 h)	63.7 ± 5.6	39.2 ± 4.6	0.001	49.5 ± 4.8	53.5 ± 6.2	NS
Plasma insulin (ng/L × 3 h)	87.8 ± 9.3	57.8 ± 6.1	0.008	80.8 ± 9.2	64.8 ± 7.1	NS
Plasma GIP (ng/L × 3 h)	41.3 ± 3.1	29.2 ± 2.2	0.0001	36.5 ± 2.7	33.9 ± 2.6	NS
Plasma GLP-1 (pg/L × 3 h)	826 ± 83	564 ± 81	0.05	664 ± 81	726 ± 83	NS

[1]Data are reported as incremental glucose AUC during the 3-h test period. All values are means ± SEMs, $n = 19$ (5 men/14 women). GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; HAW, high-amylose wheat; LAW, low-amylose wheat; NS, not significant.

Glycemic response of study participants to glucose drink

The mean glucose AUC response for the glucose beverage was 112 ± 15 mmol/L × 3 h. The maximum rise in plasma glucose concentration from baseline was 2.3 ± 0.2 mmol/L, and the time to reach maximum plasma glucose concentration was 26 ± 2 min.

Amylose concentration of bread altered glycemic, insulinemic, and incretin response

HAW breads resulted in a 39% lower glycemic AUC response (**Table 3**) and 24% lower insulinemic AUC response (Table 3) than the LAW breads. The reduction in glycemic response to the HAW breads was most pronounced at 30 min when the rise in plasma glucose concentration was substantially smaller than for LAW-W bread (**Figure 2**A; $P < 0.05$). The insulinemic response was only lower for HAW-W at 60 and 120 min and HAW-R at 120 min compared with LAW-R (Figure 2B). The maximum rise in plasma glucose concentration was 33% less when the HAW breads were consumed (1.2 ± 0.1 mmol/L) compared with those made from LAW (1.8 ± 0.1 mmol/L; $P < 0.01$), but the time to reach maximum plasma glucose concentration was similar for HAW (40 ± 3 min) and LAW breads (34 ± 3 min; $P = 0.087$).

At each time point during the 3-h test period, the plasma GIP and GLP-1 concentrations were similar when all test breads were consumed (Figure 2C, D). However, the consumption of HAW breads resulted in a 30% lower GIP and GLP-1 IAUC compared with the LAW breads (Table 3).

The glycemic index for the LAW-R was 70 and could not be calculated for the other test breads as the servings provided contained <50 g of available carbohydrate.

Refinement of flour did not influence glycemic, insulinemic, and incretin responses

Consumption of the wholemeal and refined flour breads resulted in similar glycemic and insulinemic responses (Table 3). The incretin response (GIP and GLP-1) was also similar following wholemeal and refined flour bread consumption (Table 3).

Amylose content did not affect satiety

During the postprandial period subjective satiety was similar after the 4 test breads were consumed ($P > 0.05$) and was not affected by flour processing or amylose content (**Figure 3**). However, measures of subjective satiety for some of the test breads were higher than the glucose drink during the postprandial period (Figure 3). At 30 min, satiety was higher following consumption of all test breads compared with the glucose drink (all $P < 0.05$). At 60 min, satiety remained higher for LAW-W bread compared with the glucose drink ($P < 0.01$), but was similar for the other test breads. At 120 and 180 min, satiety was higher than glucose for LAW-W, HAW-W, and HAW-R.

Subjective craving measures during the postprandial period were similar following consumption of the four test breads (all $P > 0.05$) (Figure 3). Additionally, flour processing and amylose content had no effect on craving (all $P > 0.05$). However, measures of subjective craving for some of the test breads were lower than for the glucose drink during the first 60 min of the postprandial period (Figure 3). At 30 min, cravings were lower following consumption of the wholemeal breads compared with the glucose drink (all $P < 0.05$). At 60 min, only LAW-W elicited a lower amount of craving than the glucose drink ($P < 0.05$).

Plasma ghrelin concentrations decreased following test bread consumption, reaching nadir after 60 min, before returning to concentrations above baseline by 180 min ($P < 0.0001$) (**Figure 4**). The amylose content and flour processing (wholemeal or refined) did not differentially affect the ghrelin response of the test breads (Figure 4).

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

Plasma ICAM-1 and nitrotyrosine concentrations were not differentially affected by the amylose content of the bread or flour processing (wholemeal or refined) (**Supplemental Figure 1**).

Upper gut intestinal transit rate

The time taken for the test foods to reach the large bowel did not differ among the LAW-R (352 ± 35 min), LAW-W (292 ± 33 min), HAW-R (290 ± 9 min), or HAW-W test breads (270 ± 21 min) ($P > 0.05$).

Discussion

The current study has demonstrated that substitution of conventional LAW flour with HAW flour lowered the postprandial glycemic response of bread by 39% and the insulinemic response by 24%. This was consistent with the lower circulating concentrations of the incretin hormones, GIP and GLP-1, which are secreted in response to the presence of glucose in the small intestine to stimulate insulin release from pancreatic β cells. The HAW-induced dampening of postprandial glycemia is most likely due to the reduced amount and availability of carbohydrate in these breads compared with control (conventional) breads, such that the HAW breads contain 20–40% less carbohydrate and up to 4.3% more RS, primarily due to the higher amylose concentration. The compact structure of

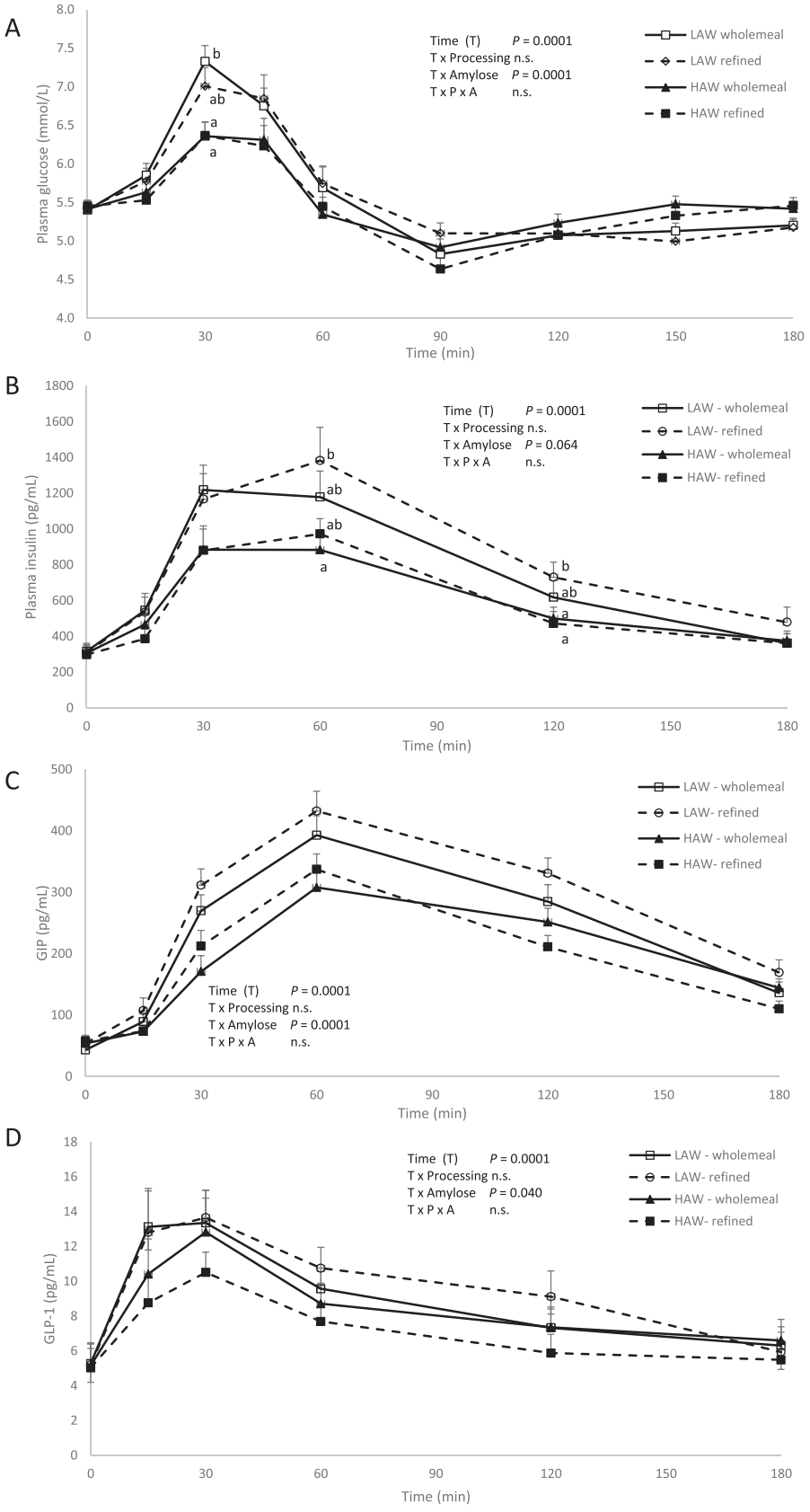

FIGURE 2 Metabolic response in healthy adults after consumption of breads that contain wheat flour that differs in amylose content and amount of flour processing: plasma glucose (A), plasma insulin (B), plasma GIP (C), and serum GLP-1 (D). Labeled means at a time without a common letter differ, $P \leq 0.05$. All values are means ± SEMs, $n = 19$ (5 men/14 women). GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; HAW-R, high-amylose wheat refined; HAW-W, high-amylose wheat wholemeal; LAW-R, low-amylose wheat refined; LAW-W, low-amylose wheat wholemeal; NS, not significant.

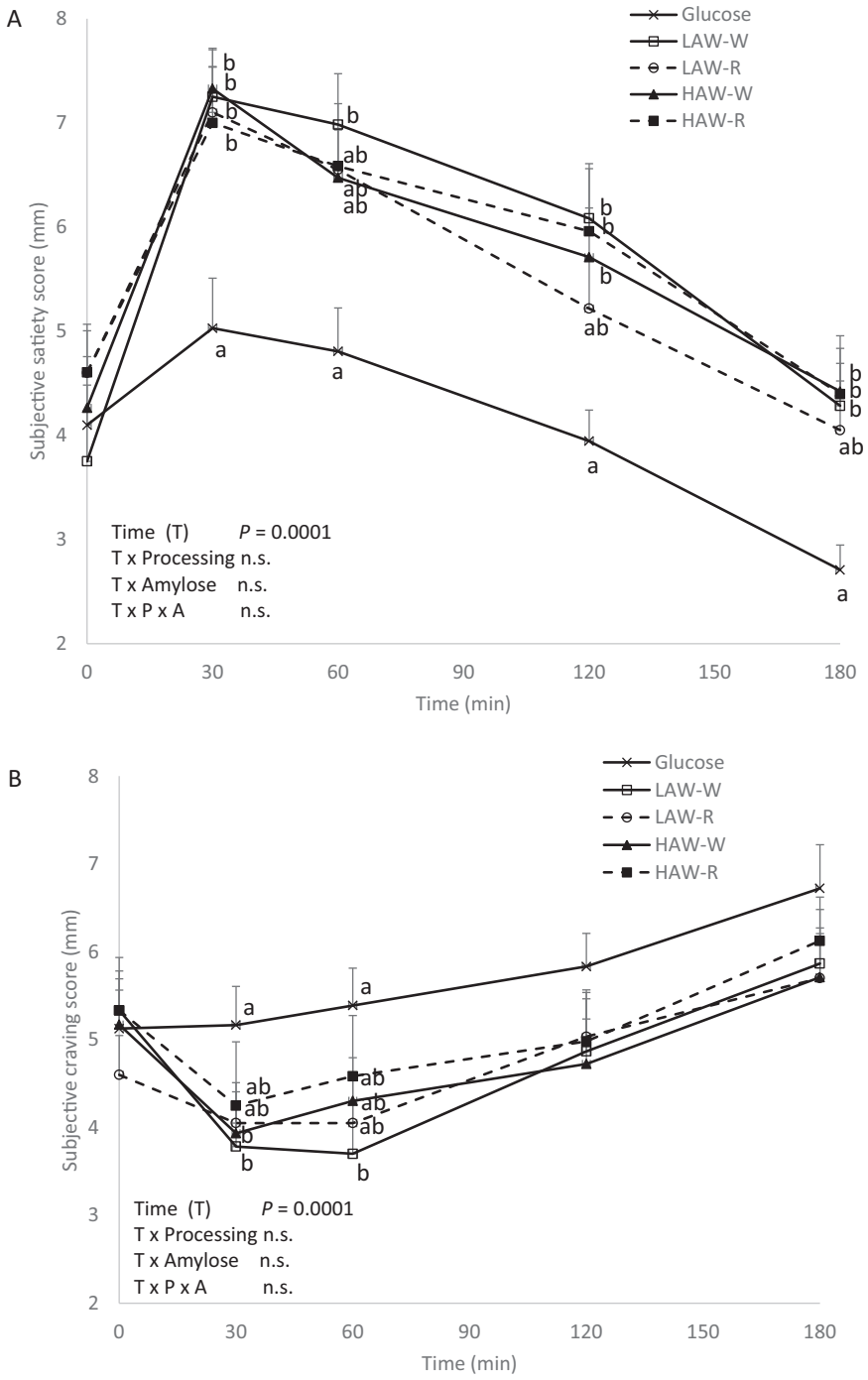

FIGURE 3 Changes in subjective satiety (A) and cravings (B) of healthy adults after consumption of breads that contain wheat flour that differs in amylose content and amount of flour processing. All values are means ± SEMs, $n = 19$ (5 men/14 women). Labeled means at a time without a common letter differ, $P \leq 0.05$. HAW-R, high-amylose wheat refined; HAW-W, high-amylose wheat wholemeal; LAW-R, low-amylose wheat refined; LAW-W, low-amylose wheat wholemeal; n.s., not significant.

starch with elevated amylose content may also restrict starch swelling and gelatinization, reducing the rate and extent of digestion (12, 24), and changes in the amylopectin structure in HAW could also contribute to the reduced starch digestion rates. Furthermore, the similar glycemic 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 RS; compared with HAW-R, HAW-W had less starch (refined 28.5 g/100 g, wholemeal 19.9 g/100 g) and less of this starch was RS (refined 4.7 g/100 g, wholemeal 3.2 g/100 g). Importantly, this study demonstrates that substitution of conventional wheat flours with either refined or wholemeal wheat could be an effective approach to lower the glycemic response of bread.

The lower glycemic and insulinemic responses 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 concentrations of amylose (5–8, 10, 11, 25). Arepas made

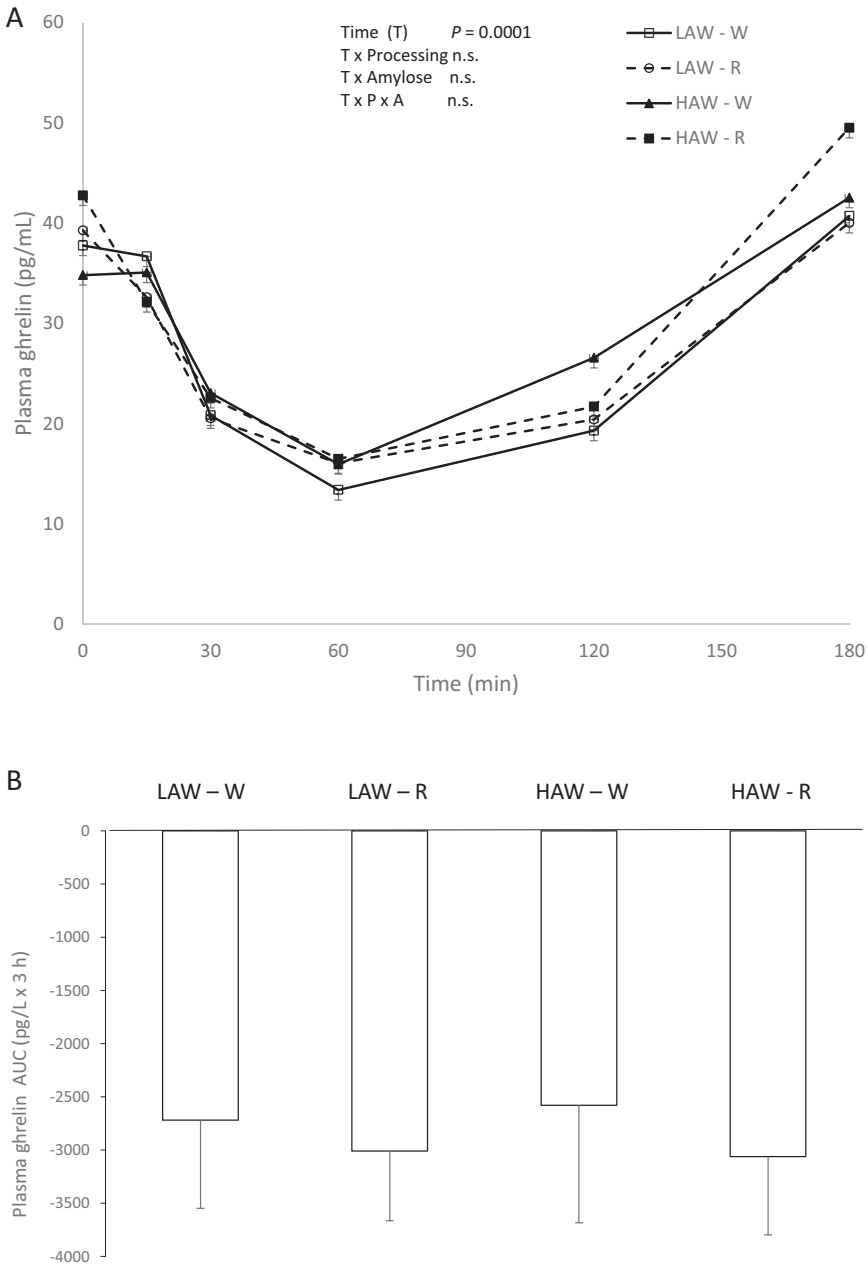

FIGURE 4 Ghrelin responses (A) and AUC (B) after healthy adults consumed breads that contain wheat flour that differs in amylose content and amount of flour processing. All values are means ± SEMs, $n = 19$ (5 men/14 women). HAW-R, high-amylose wheat refined; HAW-W, high-amylose wheat wholemeal; LAW-R, low-amylose wheat refined; LAW-W, low-amylose wheat wholemeal; n.s., not significant.

from high-amylose corn starch (70% amylose) were found to elicit a smaller glycemic and insulinemic response than those made from conventional corn starch (25% amylose) (13). A smaller glycemic and insulinemic response in men and women was observed when the wheat flour in bread was replaced with high-amylose maize starch. Importantly, amylose had to comprise at least 50% of total starch for this functional effect to occur (13). When bread was made from wholegrain wheat containing only slightly elevated concentrations of amylose (38%), the glycemic response was no different to that of bread made from conventional wholegrain wheat (17).

Substitution of conventional wheat with HAW in commonly eaten foods could contribute to both the prevention and management of type 2 diabetes. In support of this, a study in adults with either prediabetes or type 2 diabetes showed that consumption of rice containing RS for 4 wk reduced fasting insulin and insulin resistance (as calculated by HOMA-IR), and glucose and insulin areas under the response curve after a standard meal (26). Although other studies have reported that insulin sensitivity was not affected by RS-containing foods, this may be due to differences in the test foods (e.g., food form, type of processing, amount and type of RS, and study duration) or the high glucose tolerance of the healthy participants used in the studies (27–31). The most likely mechanism whereby long-term consumption of foods high in RS improves insulin sensitivity is through the lowered amount of insulin needed to stimulate glucose uptake. A study by Dainty et al. (32) 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, and at the same time improved the fasting insulin sensitivity in adults at risk of diabetes. An earlier study by Behall et al. (27) also showed that consumption of foods containing high-amylose corn starch for 5 wk reduced glycemic and insulinemic postprandial responses.

In the current study, processing of flour had little effect on glycemic response despite the wholemeal breads having more than double the fiber content and up to 30% less starch than breads made from refined flour. Previous studies have shown that white and wholemeal breads have similar postprandial glycemic responses in both diabetic and healthy adults (33–35). In addition, conventional markers of glycemic control were similar when wholegrain or refined-grain products (predominantly bread made with milled whole wheat) were consumed by volunteers for 6–12 wk (36, 37). Unlike oat and barley, the fiber content of wheat is predominantly insoluble fiber, which has limited influence on glycemic impact. Furthermore, the use of oat and barley flours to make unleavened bread is limited because these flours, when added in quantity, adversely affect the dough quality. Other strategies are therefore required to further attenuate starch digestion or absorption (38).

Certain test breads in the current study elicited increased satiety and reduced food cravings compared with the glucose control; however, there were no differences between the 4 breads. This suggests that the amylose content of the breads and the flour processing have no bearing on these endpoints and is consistent with a previous acute study showing that breakfast meals containing RS and pullulan lowered postprandial glucose, insulin, and GLP-1 responses but had no effect on satiety (8). Although other studies have shown that subjective appetite ratings improve when a test bread or muffin contained high-amylose corn starch and another fiber such as medium-weight guar gum or maltitol (25, 39), a study by Willis et al. (40) showed that muffins containing high concentrations of RS (8 g/serving) suppressed some satiety measures, including hunger and the desire to eat, more than the control low-fiber muffin. Given the lack of consistency in these findings, more studies are required to better understand the food applications whereby RS increases satiety and reduces food cravings.

The HAW breads provided 6–9% less energy (in kJ/100 g) than the conventional (LAW) control breads used in this study. Although the lower energy density and higher fiber content of the high-amylose breads did not influence satiety or the desire to eat, postprandial blood ghrelin concentration, or small intestinal transit rate, foods made from HAW flour could still potentially lower total daily energy intake and contribute to long-term weight management, and this possibility deserves further investigation (41).

Markers of inflammation and oxidative stress remained unchanged during the postprandial period, which was surprising given that a comparable study showed significant changes in these markers (42). Further examination of these and other related markers of inflammation and oxidative stress are warranted in studies of longer duration given the high concentrations of phytochemicals in whole grains which may have enhanced bioavailability following intestinal fermentation (43), and given that a range of studies on wholegrain consumption lasting 4–8 wk show improvements in some measures of inflammation (44–46).

Although the long-term effects of RS have been consistently demonstrated to improve glucose control, this could not be evaluated because 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, and 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. It was also not possible to determine whether the glycemic response differed between men and women due to the low number of total participants and larger proportion of females. Because glycemic testing was not standardized based on menstrual cycle phase, the variability in the glycemic response may have been greater given previous research (47).

In conclusion, in healthy nondiabetic men and women the postprandial rise in blood glucose elicited by the consumption of HAW bread was less than following the consumption of conventional LAW bread. The lower glycemic response with HAW bread was predominantly due to the reduced amount and availability of carbohydrate in these breads, which resulted in lower incretin and insulinemic responses compared with conventional wheat bread. Importantly, the smaller glycemic and insulinemic responses for HAW bread were similar whether it was made from wholemeal or refined flour. Although this study has only evaluated the glycemic response of breads made from HAW flour, high-amylose flour in other wheat-based food products, such as ready-to-eat breakfast cereals and bars, noodles, and doughs, may lower the glycemic impact of a range of different foods and deserves further investigation. Furthermore, it is important to evaluate whether prolonged consumption of bread and other foods containing HAW could assist in improving glucose control in healthy individuals and also in adults with prediabetes. These findings are particularly pertinent for the global population given the high glycemic nature of many foods made from conventional LAW, a staple for a majority of countries challenged with the diabetes epidemic.

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References

1. Richardson DP. Wholegrain health claims in Europe. Proc Nutr Soc 2003;62(1):161–9.

2. Slavin JL. Mechanisms for the impact of whole grain foods on cancer risk. J Am Coll Nutr 2000;19(3 Suppl):300S–7S.
3. Venn BJ, Mann JI. Cereal grains, legumes and diabetes. Eur J Clin Nutr 2004;58(11):1443–61.
4. Koh-Banerjee P, Franz M, Sampson L, Liu S, Jacobs DR, Jr, Spiegelman D, Willett W, Rimm E. Changes in whole-grain, bran, and cereal fiber consumption in relation to 8-y weight gain among men. Am J Clin Nutr 2004;80(5):1237–45.
5. Granfeldt Y, Drews A, Bjorck I. Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. J Nutr 1995;125(3):459–65.
6. Behall KM, Scholfield DJ. Food amylose content affects postprandial glucose and insulin responses. Cereal Chem 2005;82(6):654–9.
7. Al-Tamimi EK, Seib PA, Snyder BS, Haub MD. Consumption of cross-linked resistant starch (RS4(XL)) on glucose and insulin responses in humans. J Nutr Metab 2010;2010, 1–6. doi:10.1155/2010/651063.
8. Klosterbuer AS, Thomas W, Slavin JL. Resistant starch and pullulan reduce postprandial glucose, insulin, and GLP-1, but have no effect on satiety in healthy humans. J Agric Food Chem 2012;60(48): 11928–34.
9. Granfeldt Y, Liljeberg H, Drews A, Newman R, Bjorck I. Glucose and insulin responses to barley products: influence of food structure and amylose–amylopectin ratio. Am J Clin Nutr 1994;59(5): 1075–82.
10. King RA, Noakes M, Bird AR, Morell MK, Topping DL. An extruded cereal made from a high amylose barley cultivar has a low glycemic index and lower plasma insulin response than one made from a standard barley. J Cereal Sci 2008;48:526–30.
11. Aldughpassi A, Abdel-Aal E-SM, Wolever TMS. Barley cultivar, kernel composition, and processing affect the glycemic index. J Nutr 2012;142(9):1666–71.
12. Akerberg A, Liljeberg H, Bjorck I. Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. J Cereal Sci 1998;28(1):71–80.
13. Behall KM, Hallfrisch J. Plasma glucose and insulin reduction after consumption of breads varying in amylose content. Eur J Clin Nutr 2002;56(9):913–20.
14. Goddard MS, Young G, Marcus R. The effect of amylose content on insulin and glucose responses to ingested rice. Am J Clin Nutr 1984;39(3):388–92.
15. Miller JB, Pang E, Bramall L. Rice—a high or low glycemic index food? Am J Clin Nutr 1992;56(6):1034–6.
16. Yamamori M, Endo TR. Variation of starch granule proteins and chromosome mapping of their coding genes in common wheat. Theor Appl Genet 1996;93(1–2):275–81.
17. Hallstrom E, Sestili F, Lafiandra D, Bjorck I, Ostman E. A novel wheat variety with elevated content of amylose increases resistant starch formation and may beneficially influence glycaemia in healthy subjects. Food Nutr Res 2011;55, 1–8. doi:10.3402/fnr.v55i0.7074.
18. Regina A, Berbezy P, Kosar-Hashemi B, Li SZ, Cmiel M, Larroque O, Bird AR, Swain SM, Cavanagh C, Jobling SA, et al. A genetic strategy generating wheat with very high amylose content. Plant Biotechnol J 2015;13(9):1276–86.
19. Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, Wolever TM. Glycaemic index methodology. Nutr Res Rev 2005;18(1):145–71.
20. McCleary BV, Monaghan DA. Measurement of resistant starch. J AOAC Int 2002;85:665–75.
21. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24(1):38–48.
22. Rebello CJ, Johnson WD, Martin C, Johnson J, O'Shea M, Chu Y, Greenway FL. Effect of two oat-based cereals on subjective ratings of appetite. Curr Top Nutraceutical Res 2018;16:113–20.
23. Read NW, Miles CA, Fisher D, Holgate AM, Kime ND, Mitchell MA, Reeve AM, Roche TB, Walker M. Transit of a meal through the stomach, small intestine, and colon in normal subjects and its role in the pathogenesis of diarrhea. Gastroenterology 1980;79(6): 1276–82.
24. Panlasigui LN, Thompson LU, Juliano BO, Perez CM, Yiu SH, Greenberg GR. Rice varieties with similar amylose content differ in starch digestibility and glycemic response in humans. Am J Clin Nutr 1991;54(5):871–7.
25. Ekstrom LM, Bjorck IM, Ostman EM. On the possibility to affect the course of glycaemia, insulinaemia, and perceived hunger/satiety to bread meals in healthy volunteers. Food Funct 2013;4(4): 522–9.
26. Kwak JH, Paik JK, Kim HI, Kim OY, Shin DY, Kim H-J, Lee JH, Lee JH. Dietary treatment with rice containing resistant starch improves markers of endothelial function with reduction of postprandial blood glucose and oxidative stress in patients with prediabetes or newly diagnosed type 2 diabetes. Atherosclerosis 2012;224(2): 457–64.
27. Behall KM, Scholfield DJ, Yuhaniak I, Canary J. Diets containing high amylose vs amylopectin starch: effects on metabolic variables in human subjects. Am J Clin Nutr 1989;49(2):337–44.
28. Behall KM, Howe JC. Effect of long-term consumption of amylose vs amylopectin starch on metabolic variables in human subjects. Am J Clin Nutr 1995;61(2):334–40.
29. Weickert MO, Mohlig M, Koebnick C, Holst JJ, Namsolleck P, Ristow M, Osterhoff M, Rochlitz H, Rudovich N, Spranger J, et al. Impact of cereal fibre on glucose-regulating factors. Diabetologia 2005;48(11):2343–53.
30. Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 2005;82(3):559–67.
31. Noakes M, Clifton PM, Nestel PJ, Le Leu R, McIntosh G. Effect of high-amylose starch and oat bran on metabolic variables and bowel function in subjects with hypertriglyceridemia. Am J Clin Nutr 1996;64(6):944–51.
32. Dainty SA, Klingel SL, Pilkey SE, McDonald E, McKeown B, Emes MJ, Duncan AM. Resistant starch bagels reduce fasting and postprandial insulin in adults at risk of type 2 diabetes. J Nutr 2016;146(11):2252–9.
33. Jenkins DJ, Wolever TM, Jenkins AL, Giordano C, Giudici S, Thompson LU, Kalmusky J, Josse RG, Wong GS. Low glycemic response to traditionally processed wheat and rye products: bulgur and pumpernickel bread. Am J Clin Nutr 1986;43(4):516–20.
34. Scazzina F, Del Rio D, Pellegrini N, Brighenti F. Sourdough bread: starch digestibility and postprandial glycemic response. J Cereal Sci 2009;49(3):419–21.
35. Kristensen M, Jensen MG, Riboldi G, Petronio M, Bugel S, Toubro S, Tetens I, Astrup A. Wholegrain vs. refined wheat bread and pasta. Effect on postprandial glycemia, appetite, and subsequent ad libitum energy intake in young healthy adults. Appetite 2010;54(1): 163–9.
36. Jenkins DJ, Kendall CW, Augustin LS, Martini MC, Axelsen M, Faulkner D, Vidgen E, Parker T, Lau H, Connelly PW, et al. Effect of wheat bran on glycemic control and risk factors for cardiovascular disease in type 2 diabetes. Diabetes Care 2002;25(9): 1522–8.
37. Andersson A, Tengblad S, Karlstrom B, Kamal-Eldin A, Landberg R, Basu S, Aman P, Vessby B. Whole-grain foods do not affect insulin sensitivity or markers of lipid peroxidation and inflammation in healthy, moderately overweight subjects. J Nutr 2007;137(6): 1401–7.
38. Scazzina F, Siebenhandl-Ehn S, Pellegrini N. The effect of dietary fibre on reducing the glycaemic index of bread. Br J Nutr 2013;109(7):1163–74.
39. Quilez J, Bullo M, Salas-Salvado J. Improved postprandial response and feeling of satiety after consumption of low-calorie muffins with maltitol and high-amylose corn starch. J Food Sci 2007;72(6): S407–11.
40. Willis HJ, Eldridge AL, Beiseigel J, Thomas W, Slavin JL. Greater satiety response with resistant starch and corn bran in human subjects. Nutr Res 2009;29(2):100–5.
41. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev 2001;81(3):1031–64.
42. Dickinson S, Hancock DP, Petocz P, Ceriello A, Brand-Miller J. High-glycemic index carbohydrate increases nuclear factor-kappaB activation in mononuclear cells of young, lean healthy subjects. Am J Clin Nutr 2008;87(5):1188–93.
43. Belobrajdic DP, Bird AR. The potential role of phytochemicals in wholegrain cereals for the prevention of type-2 diabetes. Nutr J 2013;12:62.

44. Roager HM, Vogt JK, Kristensen M, Hansen LBS, Ibrugger S, Maerkedahl RB, Bahl MI, Lind MV, Nielsen RL, Frokiaer H, et al. Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. 2019;68(1):83–93.
45. Vanegas SM, Meydani M, Barnett JB, Goldin B, Kane A, Rasmussen H, Brown C, Vangay P, Knights D, Jonnalagadda S, et al. Substituting whole grains for refined grains in a 6-wk randomized trial has a modest effect on gut microbiota and immune and inflammatory markers of healthy adults. Am J Clin Nutr 2017;105(3):635–50.
46. Vitaglione P, Mennella I, Ferracane R, Rivellese AA, Giacco R, Ercolini D, Gibbons SM, La Storia A, Gilbert JA, Jonnalagadda S, et al. Whole-grain wheat consumption reduces inflammation in a randomized controlled trial on overweight and obese subjects with unhealthy dietary and lifestyle behaviors: role of polyphenols bound to cereal dietary fiber. Am J Clin Nutr 2015;101(2):251–61.
47. Brown SA, Jiang B, McElwee-Malloy M, Wakeman C, Breton MD. Fluctuations of hyperglycemia and insulin sensitivity are linked to menstrual cycle phases in women with T1D. J Diabetes Sci Technol 2015;9(6):1192–9.