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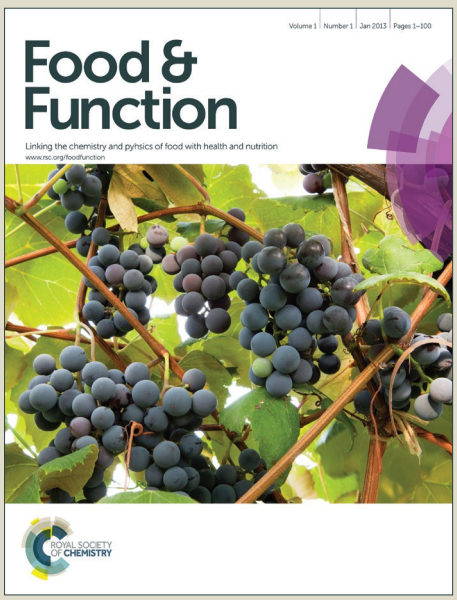

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Improved course of glycaemia after a bread based breakfast is associated with beneficial effects on acute and semi-acute markers of appetite

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Abstract

The prevalence of type 2 diabetes mellitus is rapidly increasing all over the world and a diet promoting reduced glycaemic excursions in the postprandial phase may help to prevent the disease.

In the present study guar gum (GG) and whole grain rye flour or high amylose maize starch (HAM) was combined to design bread products giving low and sustained glycaemia. A meal study was performed with young, healthy subjects and in addition to glucose and insulin, also subjective appetite ratings and biomarkers of appetite, voluntary energy intake at a second meal and markers of fermentation were studied. The combination of GG and rye was superior with improvements in subjective appetite whereas both test products lead to improvements in biomarkers of appetite compared to the white wheat bread reference. The inclusion of GG, rye and/or HAM in bread products show great potential in lowering risk factors associated with insulin resistance and improving acute and semi-acute appetite.

Background

The prevalence of type 2 diabetes mellitus (T2DM) is rapidly increasing all over the world.[1] A recent review acknowledged the importance of diet and lifestyle modifications in prevention of T2DM.[2] More specifically, the importance of a diet leading to reduced glycaemic excursions in the postprandial phase has been identified as a prerequisite in order to maintain metabolic health and prevent T2DM, overweight and cardiovascular disease (CVD).[3]

Lower glycaemic excursions can be achieved by a conscious choice of ingredients in carbohydrate rich foods or meals. Both physiological factors and inherent food properties, e.g. enzymatic availability, botanical, physical or chemical structure of the food,[4, 5] presence of

certain dietary proteins[6] and/or indigestible or slowly digestible carbohydrates[7, 8] are of importance for the glycaemic response. However, the metabolic response to a meal is not only affected by its type and composition, but also by previous food intake.[8-10]

Glycaemic index (GI) is used to rank the glycaemic effect of carbohydrate rich foods during the first 2h after a meal, and low GI´s represent lower glycaemic excursions. In order to take into account also the course of glycaemia beyond 120 min, we recently introduced the concept of glycaemic profile (GP). Consequently, GP considers the duration of the glucose response and the incremental peak.[11] Based on previous findings for products with low GI and high GP[11-13] it is hypothesised that carbohydrate rich foods with a low but sustained net increment in glycaemic response, *i.e.* low GI and high GP, induce metabolic benefits both acutely and at a subsequent meal.

Rye products have repeatedly shown to lower insulin responses, regardless of their glycaemic responses[14, 15]. When comparing five different rye varieties grown in Sweden,[16] Visello rye was one of the more promising candidates to lower both postprandial glycaemia and insulinaemia. Furthermore, rye appears to promote colonic fermentative activity at an earlier point in time than other cereals.[17, 18]

Guar gum (GG) was recently shown to increase GP of bread at three different inclusion levels[13] and the suggested mechanism is by increasing viscosity in the upper small intestine.[19] The same study showed that a combination of GG and whole grain high amylose maize starch (HAM) in bread resulted in a pronounced formation of RS.[13] RS is assumed to increase colonic fermentation at a somewhat later stage during the digestion, than rye.[20] An increased amylose content also leads to formation of a slowly digestible starch fraction that affects the course of glycaemia.[21] However, at equivalent available starch basis, an increased RS-level did not influence the acute glycaemia *per se*.[13]

We hypothesized that food products modulated to give a low but sustained net increment in glycaemia (low GI/high GP) and promote early gut fermentation will lower risk factors associated with insulin resistance and improve acute and semi-acute appetite. Thus, in addition to glucose and subjective appetite ratings, we studied insulin, biomarkers of appetite, voluntary energy intake at a second meal and markers of fermentation after bread meals containing GG and either HAM or whole grain Visello rye.

Methods

Raw materials and recipes

HAM (Hi-Maize) was obtained from Ingredion Incorporated (Bridgewater, NJ, USA), medium molecular weight GG (MEYPRODOR®50) was kindly provided by Danisco A/S (Denmark) and dry yeast was obtained from Jästbolaget AB (Sollentuna, Sweden). Rye kernels (Visello) were obtained from KWS LOCHOW GMBH (Bergen, Germany). White wheat bread (WWB) was made from wheat flour with 10% protein (Vetemjöl, Kungsörnen AB, Järna, Sweden). The breads with HAM and GG (HG) and Visello rye whole grain flour and GG (VG), respectively, were made from wheat flour with 12% protein (Vetemjöl special, Kungsörnen AB, Järna, Sweden) to improve loaf volume. The Visello rye kernels were milled to whole grain flour using a laboratory mill (Perten laboratory mill 120, sieve 0.8 mm) before baking.

The WWB was made in a home baking machine (Tefal, home bread) using a program for white bread as previously described.[13] The HG and VG breads were made with a uniform procedure where the dough was mixed in a bowl for 5 min, proofed in a home baking machine (Tefal, home bread) for 30 min, kneaded for 15 s by hand and placed in the bread

machine for another 30 min proofing followed by 60 min baking. The recipes are presented in Table 1.

After baking, WWB and HG breads were left to cool for 2 h wrapped in a towel, whereas the VG breads were left for 16-18 h wrapped in a towel in a plastic bag. Thereafter, the crust was removed, the crumb sliced and portions wrapped in aluminium foil, put into plastic bags and stored in a freezer (-18ºC) until use. The day before usage, either for analyses or in the meal study, bread portions were taken from the freezer and thawed at ambient temperature, still wrapped in aluminium foil and in the plastic bag.

Composition of the lunch

In order to measure the voluntary energy intake, an *ad libitum* meal was served at 240 min after the start of the breakfast. The ordinary Swedish lunch meal consisted of regular spaghetti made from durum wheat and normal wheat (Barilla Sweden AB, Filipstad, Sweden), ready-made frozen meatballs (ICA Handlarnas AB, Solna, Sweden), ketchup (Heinz) and fresh cucumber. The cucumber was served in slices, 2-3 mm thick, with the ends removed in order for all slices to have the same ratio of peel to fruit flesh. The pasta was boiled for 8 min (1 l water, and 7 g NaCl per 100 g pasta) the water was then discarded and 8 g rape seed oil (Di Luca & Di Luca AB, Stockholm, Sweden) added per 100 g dry pasta. The meatballs were heated in a microwave oven at 850 W in 2 min cycles until they were evenly warm.

Chemical analysis

Prior to the analysis of available and total starch[22] the bread samples were air dried and milled to pass through a 0.5 mm screen (Cyclotec, Tecator, Höganäs, Sweden). Measurements of RS[23], rate of starch hydrolysis[24] and fluidity[13] were performed on the product "as is".

Available starch content of the servings was calculated by subtracting RS from total starch. The chemical characteristics of the breads are shown in Table 2. The energy content of the three test meals was calculated based on available carbohydrates (analysed) and estimated fat and protein contents using 17 kJ per g protein and available carbohydrates, and 37 kJ per g fat. The composition of the test breads are presented in Table 3, with the amount of HAM and GG estimated from the recipes and weight of bread loafs before and after baking.

Study design

Nineteen healthy non-smoking volunteers (9 men and 10 women) aged 27.3 ± 1.4 years (mean ± SEM) with normal body mass indices (21.7 ± 0.4 kg/m^2) and without drug therapy, participated in the study. All subjects had normal fasting blood glucose concentrations (5.4 ± 0.06 mmol/l). The recruitment of test subjects and the study trials were performed from September to December 2011. All test subjects gave their informed consent and were aware of the possibility of withdrawing from the study at any time. Approval of the study was obtained by the regional ethical review board in Lund, Sweden (registration number 2011/507). The subjects were instructed to maintain their regular life-style throughout the entire study. The day prior to a test the participants were told to avoid alcohol, excessive physical activity and food rich in dietary fibre (DF). In the late evening (21.00-22.00) prior to a test the subjects were instructed to eat a standardized meal consisting of white wheat bread with topping and drink of their own choice. However, the subjects were obliged to have an identical evening meal before each test. The test and reference products were provided as breakfast meals in random order approximately one week apart. The subjects arrived in the laboratory at 07.45 on the test day after an overnight fast. A peripheral venous catheter (BD Venflon Dickinson, Helsingborg, Sweden) was inserted into an antecubital vein. Capillary plasma glucose and venous blood samples were taken in the fasting state, after which the test

meals, contributing with 50 g of available starch, were served with 250 g of tap water (time 0). The subjects were told to finish the meal within 14 min. Blood samples were then taken at 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after the beginning of the breakfast. After the blood sampling at 120 min, 150 ml coffee, tea or water was served. The same drink was then used for each participant throughout the study. After the sampling at 240 min the lunch meal was served in a buffet style. Further (venous) blood samples were taken at 300 and 360 min after breakfast. The participants were told to eat until they were pleasantly full and try to reach the same level of satiation at every test occasion. Therefore they were allowed to take the food by themselves and the amount of food was recorded by the study leader. Water (250 ml) was served with the lunch meal. The subjects were asked to rate their subjective *feeling of hunger*, *satiety* and *desire to eat* on a bipolar visual analogue scale (VAS) directly after each blood sampling. During the experiment the subjects were not allowed to eat or drink anything except for the food provided and they were told to remain seated as much as possible.

Blood analysis

Plasma glucose concentrations were determined in capillary whole blood at all time points before lunch using a HemoCue Glucose 201+ Analyser (HemoCue AB, Ängelholm, Sweden). Serum samples were collected in 3.5 ml SST tubes and plasma samples in 2.0 ml EDTA tubes pre-treated with inhibition mix (2 mg Pefablock (Roches) and 20µl DPPIV (Millipore) in each test tube). The inhibition mix was added to each tube by a syringe no more than 4 days before the usage and the tubes were then stored in 8°C. Tubes for serum were centrifuged for 10 min (2000 G, 4°C) after 30 min of clotting. Test tubes for plasma were kept on ice before and after sampling and these tubes were centrifuged for 10 min (1000 G, 4°C) as soon as possible. Blood samples were then frozen in aliquots at -18ºC until analysis.

NEFA was measured in serum at 180 and 240 min by an enzymatic colorimetric method (NEFA C, ACS-ACOD method, WAKO Chemicals GmbH, Germany).

Insulin, ghrelin (active), GIP (total) and PYY (PYY_{1-36} and PYY_{3-36}) were measured by MILLIPLEX MAP (Human Metabolic Hormone Magnetic Bead Panel, Millipore Corporation, Billerica, MA, USA) at all time points.

As an indicator of colonic fermentation, breath hydrogen (H_2) excretion was measured every 30 min during the entire test day using a Gastrolyser (Bedfont EC60 Gastrolyser, Rochester, UK). Short chain fatty acids (SCFA - acetate, propionate, isobutyrate and butyrate) in serum were analysed at 180, 240, 300 and 360 min using gas chromatography.[25]

Calculations and statistical methods

Data are expressed as least square means (LSMs) and standard errors of the mean (SEM). One subject was not able to finish the VG portion so the data was analysed with n_{WWB} and n_{HG} = 19 and n_{VG} = 18.

The incremental- and total areas under the curves (iAUC and tAUC, respectively) were calculated for each subject and test meal using the trapezoid model. GI and insulinaemic index (II) were calculated from the iAUC 0-120 min for glucose and insulin respectively, using WWB as the reference (GI and II = 100). HI was calculated from tAUC 0-180 min using WWB as the reference. The predicted GI was calculated from HI as described by Leeman *et al.*[26] The result for fluidity index (FI) was calculated as: $(\text{consistency}_{\text{reference bread}})/(\text{consistency}_{\text{test bread}})\times 100\%$, where consistency is the reciprocal of the fluidity (1/Bostwick Units (BU)) and BU indicates the flowing distance of the sample after 60 s in cm, divided by the sample size (ml).[13, 27]

Incremental peaks (iPeak) for glucose, insulin and GIP were calculated as the maximum postprandial increase from baseline. The GP was defined as the duration of the glucose curve

above fasting concentration in the timespan from breakfast to lunch (0-240 min) divided by the iPeak[11]. GraphPad Prism (version 6, GraphPad Software, San Diego, CA, USA) was used for graph plotting and area calculation.

The effect of reference and test meals on physiological responses was evaluated using a linear mixed model ANCOVA (PROC MIXED procedure). Baseline, visit, treatment, time and treatment x time interaction were included as fixed effects. Subject was treated as random effect and time and visit were included as repeated effects. All models were tested for the normality of residuals using standard diagnostics to ensure that all variables met the assumptions for normal distribution and ln transformation was applied if necessary (the case for insulin, ghrelin and GIP). To adjust for multiple comparisons of significant effects, Tukey-Kramer *post hoc* significance test was performed, the Kenward-Roger correction was applied for reducing small sample bias. Calculations were performed using SAS (version 9.4, SAS Institute Inc., Cary, USA).

For HI a mixed model analysis of variance (ANOVA) was used with test subject as a random variable. The same procedure was used for FI, but in this case the replicate was used as random variable (MINITAB, release 16, Minitab Inc., State College PA).

Correlation analysis was conducted to evaluate the relation among dependent measures with the use of Spearman´s partial coefficients controlling for subjects and corresponding baselines (two tailed test) (SPSS software, version 22; SPSS Inc., Chicago, IL, USA). Statistical significance was considered at a p-value < 0.05 (two-tailed).

Results

Glucose responses at breakfast

The fasting concentrations for plasma glucose did not differ between the treatments (Table 4). There was no significant treatment effect ($p = 0.16$) among the meals (Table 4), however, a

time x treatment interaction was found ($p < 0.0001$) (Fig. 1). HG and VG both resulted in significant lower GI and glucose iPeak, as well as higher GP, compared to the WWB.

Insulin and NEFA responses

The fasting concentrations for plasma insulin did not differ between the treatments (Table 4). HG and VG resulted in significantly lower overall insulin response (0-360 min, $p = 0.003$) compared to the WWB (Table 4). Furthermore, there was a significant time x treatment interaction ($p < 0.0001$) (Fig. 1). II and insulin iPeak was significant lower for HG and VG compared to the WWB, with II for HG also being significantly lower than that of VG.

Incremental insulin responses after intake of the *ad libitum* lunch meal (iAUC 240-360) was significantly lower after VG compared to HG breakfast ($p = 0.017$), whereas WWB did not differ from any of the two (WWB compared to HG $p = 0.88$ and WWB compared to VG $p = 0.067$, respectively).

VG induced a lower concentration of NEFA than WWB at 240 min ($p = 0.014$), whereas HG did not differ from any of the two products.

Ghrelin

There was no significant treatment effect for ghrelin between the meals ($p = 0.70$). However, a significant time x treatment interaction was found ($p < 0.0001$) (Fig. 2). The mean plasma ghrelin level decreased to a nadir at 54 ± 3 min, with a significantly smaller relative decrease for HG and VG compared to WWB. HG and VG had a significantly lower relative increase from the nadir to 240 min at lunch time, compared with WWB. Ghrelin at 240 min was positively correlated to the energy intake at lunch ($r = 0.297$, $p = 0.028$).

GIP

HG and VG resulted in significantly lower overall GIP responses (0-360 min, $p < 0.0001$) compared to WWB (Table 4). There was a significant time x treatment interaction for GIP ($p < 0.0001$) (Fig. 2). HG and VG resulted in significantly lower iAUC and iPeak values for GIP compared to the WWB in the timespan from breakfast to lunch.

PYY

HG and VG resulted in significantly lower overall PYY response (0-360 min, $p = 0.0002$) compared to WWB (Table 4). There was no significant time x treatment interaction ($p = 0.0938$). The tAUC in the time period after the *ad lib* lunch (tAUC 240-360) was significantly higher after the VG breakfast compared to WWB (Fig. 3).

Breath H_2 and s-SCFA

There was no significant treatment effect for breath H_2 ($p = 0.11$), however, a significant time x treatment interaction was found (0-360 min, $p = 0.008$) (Fig. 3). In the period after lunch (240-360 min), the VG breakfast tended to give a higher iAUC for H_2 compared to the WWB and HG ($p = 0.058$).

The amount of acetate, propionate and isobutyrate in serum did not differ between any of the products throughout the test day. The HG breakfast gave rise to a higher concentration of s-butyrate at 240 min compared to WWB and VG, see Table 4.

Subjective appetite ratings and energy intake at the ad libitum *lunch meal*

VG resulted in significantly lower overall *feeling of hunger* compared to the WWB in the period from breakfast to lunch (p = 0.017) (Table 5 and supplemental Fig 1), but no differences were found for *feeling of fullness* or *desire to eat*. No significant time x treatment interaction was found for *feeling of fullness, feeling of hunger* or *desire to eat* (0-240 min, p = 0.65, 0.93 and 0.41, respectively).

There were no difference in energy intake at the voluntary lunch (p = 0.087) (Table 5).

Correlations

Correlations between responses of glucose, insulin and appetite biomarkers as well as subjective appetite ratings and HI/FI are presented in Supplemental Table 1. Both glucose and insulin (iAUC 0-120) were positively correlated to NEFA (240 min), GIP (iAUC 0-120), HI and FI, and negatively correlated to ghrelin (difference nadir to 240 min) and PYY (240 min). For GP most correlations were similar but with opposite signs. Insulin (iAUC 0-120) was correlated to *feeling of satiety* (tAUC 0-240) and both insulin and glucose (iAUC 0-120) were correlated to *desire to eat* (tAUC 0-240). Correlations between subjective appetite ratings and appetite biomarkers in the period from breakfast to lunch are presented in Supplemental Table 2 and those between subjective appetite ratings, appetite biomarkers and breath hydrogen excretion after lunch in Tables 8 and 9.

Discussion

In the present study we confirm that the inclusion of 10% GG (flour basis) in bread products reduces GI and increases GP compared with white wheat reference bread. Interestingly, by combining GG with other fermentable substrates, *i.e.* rye flour or HAM, differences in appetite variables and markers of fermentation were observed. We also found correlations

between biomarkers of appetite (ghrelin and PYY) and measures of glucose and insulin (glucose iAUC 0-120, GP and insulin iAUC 0-120).

The glucose iPeak for both VG and HG was lowered by 1.3 and 1.4 mmol/l (-41 and -44%, respectively) compared to the WWB reference. Previously, a bread with similar concentrations of GG, combined with whole grain high amylose maize flour, lowered the iPeak with 1.5 mmol/l (-55%) when given in a smaller portion (37 g available carbohydrates).[13] It should be noted that both of these reductions meet the recently suggested guidelines for minimum differences in postprandial glycaemia to achieve metabolic improvements in T2DM pathogenesis.[3] Furthermore, the guidelines also emphasize the importance of a lowered insulin response and in the present study, the insulin iPeaks were significantly reduced by 29 and 37%, respectively for HG and VG, compared to WWB and the total insulin excursion was reduced by 18 and 12%, respectively. Thus, the ingredients and/or combinations could be further exploited in future development of bread products that could reduce postprandial glycaemic and insulinaemic excursions. As methods of prediction, both HI and FI were well correlated to glucose and insulin responses (iAUC 0-120). In a previous study we saw that FI and HI were better predictors of GP compared to GI.[13] This was, however, not the case in the present study where only HI correlated better to GP compared to glucose iAUC, whereas FI did not. This could possibly be a result from the inclusion of rye in the VG products, since previous observations in our lab on rye containing products indicates that the behaviour of rye in fluidity measurements is different from other cereals and GG.

In the present study we found reduced GIP-levels after the HG and VG breakfast compared to WWB, and we interpret them as reflecting a lowered gastric emptying rate (GER) caused by GG. This is in line with a study reporting lower levels of GIP and decreased GER after intake

of a high viscosity meal containing 3.3 g GG compared to a low viscosity meal without GG.[28] The present study design does, however, not allow us to isolate separate effects relating only to GG and, thus, we cannot exclude that also RS or rye could affect the GIP levels. Decreased GER can also contribute to increased satiety by prolonging the period of gastric distension after a meal.[28] The significantly higher levels of PYY after HG and VG breakfast meals were thus likely to be caused by prolonged gastric emptying and over-all transit time. Thus, the inclusion of GG, rye and/or HAM seems to be useful in the attempt to stimulate endogenous production of PYY.

The *feeling of fullness* was positively correlated to PYY-levels just before starting lunch, and at the same time the *feeling of hunger* and desire *to eat* were negatively correlated to PYY-levels. After lunch, the PYY was negatively correlated to subjective *feeling of hunger*, a correlation also reported by others.[29]

A significantly lower relative increase in ghrelin from nadir to 240 min was found after the HG and VG breakfasts compared to WWB. The ghrelin level at 240 min was positively correlated to the energy intake at lunch, which is in line with a recent review, indicating that ghrelin is an acute hunger signal in the pre-prandial period.[30]

After lunch, increased levels of breath H_2 was found following the rye containing VG breakfast indicating increased gut fermentative activity.[31] This is in line with previous studies of rye where increased H_2 excretion was found from 4 to 8 h after consumption.[12, 32] However, in the present study, the increase in H_2 excretion was not accompanied by an increase in plasma SCFA. Possibly, this could be due to the formation of other fermentation products, *e.g.* lactate, not measured here. In the present study, increased breath H_2 at 240 min was related to increased satiety and reduced hunger after lunch (240-360 min), but not to the voluntary energy intake. This could possibly indicate that the systemic effects of an increase in breath H_2 are delayed.

The HG breakfast increased the butyrate levels already after 4 h and to our knowledge this is the first study reporting such early increases in peripheral levels of a gut fermentation mediated metabolite in response to an acute meal. It has been demonstrated, though, that a late evening meal consisting of high amylose barley bread, as well as 4 weeks of rye bread consumption, prior to a wheat bread breakfast results in higher levels of butyrate and or propionate.[33, 34] No increase in SCFA was found after the consumption of VG breakfast, but preliminary data by Jakobsdottir *et al*[35] indicated an increase in SCFA around lunch time after having rye bread for breakfast. One possibility is that the current combination of rye with GG may have retained the easily fermentable rye fraction, leading to a possible delay in SCFA production beyond our studied time span. It has been hypothesised that SCFA act as a regulator of appetite and food intake through the gut-brain axis.[36] In the present study we did, however, not find any correlations between SCFA and subjective appetite or food intake at the subsequent lunch.

HAM has previously been shown to have positive effects on insulin sensitivity and fatty acid (FA) metabolism,[37] and the effect of RS on glucose tolerance can be due to mechanisms involving muscle uptake of FA. However, the lower insulin secretion following HG breakfast in the present study was not accompanied by significant reduction of NEFA. Instead it was VG that significantly lowered NEFA at the time of lunch, an effect displayed by rye products also in a previous study.[12] A prolonged digestive phase has earlier been shown to suppress the levels of NEFA in the late postprandial phase[38] and we found correlations between improved course of glycaemia (low GI/ high GP) and lower NEFA-values at 240 min. Interestingly, we also found a positive correlation between the levels of NEFA and ghrelin at lunch time. Ghrelin favours oxidation of FA as energy source[39] and this might have contributed to the increase in NEFA at lunch time after the WWB breakfast.

Conclusion

By combining GG with whole grain rye or HAM, bread products with low and sustained glycaemia were obtained. Furthermore, the combination of GG and rye stimulated PYY excretion after a subsequent *ad lib* meal. The combination of GG and rye was superior with improvements in subjective appetite. The tendency of reduced energy intake at the subsequent *ad lib* lunch warrants further investigation.

Competing interests

The authors declare no competing financial interests.

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Tables

Table 1: Ingredients in the test and reference breads.

Ingredient (g per bread)	WWB	HG	VG
Water	360	445	460
Wheat flour 10% protein	540	-	-
Wheat flour 12% protein	-	280	105
Hi-Maize (HAM)	-	160	-
Visello rye flour	-	-	360
Guar gum (GG)	-	50	55
Dry yeast	4.8	5.0	9.6
NaCl	4.8	5.0	5.0

WWB (white wheat bread), HG (bread containing HAM and GG), VG (bread containing whole grain Visello rye flour and GG).

Table 2: Chemical characteristics of test and reference bread.

Chemical characteristics	WWB	HG	VG
Total starch[1] (% of ww)	39.8	35.1	27.7
Resistant starch[2] (% of ww)	1.0	7.1	1.2
Resistant starch (% of total starch)	2.6	20.2	4.3
Dry matter content (%)	52.0	47.2	46.4
Hydrolysis index (HI)[3] %	100 a	46 ± 2 b	56 ± 3 b
Fluidity index (FI)	100 a	48 ± 1 b	27 ± 1 c
Predicted GI from HI	-	48	57

[1]Result presented as mean (n = 2), [2]result presented as mean (n = 6) [3]result presented as mean ± SEM (n = 5). Values within a column not sharing the same letters were significantly different, $p < 0.05$ (ANOVA followed by Tukey´s *post hoc* test).

383 Table 3: Composition of the breakfast meals

Composition of breakfast	WWB	HG	VG
Fresh weight (g/portion)	128.9	178.7	188.3
Energy content (kJ/portion)	1208	1074	1246
Hi-Maize (g/portion)	-	33.6	-
Guar gum (g/portion)	-	10.5	11.6
Total starch (g/portion)	51.3	62.7	52.2
Resistant starch (g/portion)	1.3	12.7	2.2

384 [1]Energy content calculated using available carbohydrates (analysed) and estimated fat and protein content.
385 Amount of Hi-maize and GG is calculated from the recipes, total starch and RS calculated from respective
386 analysis.

387

388 Table 4: Metabolic responses after intake of the test products.[1]

Test variables	Subjects (n)	WWB	HG	%[2]	VG	%[2]
Breakfast (0-240 min)						
Glucose fasting value (mmol/l)	$19_{WWB, HG,}\ 18_{VG}$	5.3 ± 0.1	5.4 ± 0.1	0	5.5 ± 0.1	3
Glucose overall mean 0-120 (mmol/l)		6.3 ± 0.1	6.1 ± 0.1	-3	6.1 ± 0.1	-4
Glucose, iPeak 0-240 (Δ mmol/l)		3.2 ± 0.2 a	1.9 ± 0.2 b	-41	1.8 ± 0.2 b	-42
GI (%)		100 a	66 ± 6 b	-35	61 ± 6 b	-39
Glucose, GP (min/mmol/l)		51 ± 10 a	95 ± 10 b	87	88 ± 11 b	75
Insulin fasting value (nmol/l)	$19_{WWB, HG,}\ 18_{VG}$	0.078 ± 0.008	0.083 ± 0.008	5	0.072 ± 0.008	-8
Insulin overall mean 0-360 (nmol/l)		$0.17 \pm 2*10^{-4}$ a	$0.14 \pm 2*10^{-4}$ b	-18	$0.15 \pm 2*10^{-4}$ ab	-10
Insulin iPeak 0-240 (Δ nmol/l)		0.35 ± 0.03 a	0.22 ± 0.03 b	-39	0.25 ± 0.04 b	-29
II (%)		100 a	44 ± 4 b	-56	59 ± 4 c	-41
Ghrelin, Δ nadir (at time 54 ± 3 min)	$19_{WWB, HG,}\ 18_{VG}$	75.5 ± 5.3 a	51.7 ± 5.3 b	-31	54.2 ± 5.4 b	-28
Ghrelin, relative increase from nadir to 240 min, (%)		54.3 ± 3.0 a	38.0 ± 3.0 b	-30	37.9 ± 3.1 b	-30
GIP overall mean 0-360 (ng/l)	$19_{WWB, HG,}\ 18_{VG}$	51.2 ± 1.1 a	38.9 ± 1.1 b	-24	40.2 ± 1.1 b	-21
GIP iPeak 0-240 (ng/l)		64.8 ± 5.1 a	36.0 ± 5.2 b	-44	34.1 ± 5.3 b	-47
GIP iAUC 0-240 (min ng/l)		7898 ± 840 a	4042 ± 840 b	-49	4219 ± 840 b	-47
PYY, overall mean 0-360 (ng/l)	$18_{WWB, HG,}\ 17_{VG}$	72.4 ± 3 a	82.3 ± 3 b	14	88.6 ± 3 b	22

NEFA (mmol/l), 240 min	$19_{WWB, HG,}$ 18_{VG}	0.28 ± 0.03 a	0.23 ± 0.03 ab	-20	0.17 ± 0.03 b	-41
Lunch (240-360 min)						
GIP iPeak 240-360 (ng/l)		168.5 ± 11.8	168.0 ± 11.4	0	163.6 ± 13.8	-3
s-Acetate 240 min (µmol/L)	$19_{WWB, HG,}$ 18_{VG}	334 ± 14	317 ± 14	-5	312 ± 15	-7
s-Propionate 240 min (µmol/L)		10.5 ± 0.4	10.8 ± 0.4	3	10.4 ± 0.4	-3
s-Isobutyrate 240 min (µmol/L)		12.0 ± 0.6 ab	12.9 ± 0.6 a	7	11.3 ± 0.6 b	-6
s-Butyrate 240 min (µmol/L)		16.1 ± 0.9 a	19.2 ± 0.9 b	19	15.8 ± 1.0 a	-2

[1]Values are LSMs ± SEM. Products in the same line not sharing the same letter are significantly different, $p < 0.05$ (ANCOVA followed by Tukey´s *post hoc* test). [2]The percent change is calculated as the difference from HG and VG to the WWB.

Table 5: Subjective appetite ratings from breakfast to lunch[1] and voluntary intake at the *ad lib* lunch.

Test variables	**WWB**	**HG**	**%[2]**	**VG**	**%[2]**
Feeling of fullness overall mean 0-240 min	35.1 ± 4	39.9 ± 4	14	44.1 ± 4	26
Feeling of hunger overall mean 0-240 min	58.6 ± 4 a	53.8 ± 4 ab	-10	47.0 ± 4 b	-20
Desire to eat overall mean 0-240 min	63.4 ± 4	57.2 ± 4	-11	54.3 ± 5	-16
Energy intake, voluntary lunch (kJ)	3586 ± 200	3603 ± 200	0	3326 ± 202	-7

[1]Products in the same line not sharing the same letter are significantly different, values are LSMs ± SEM, n = 19, (VAS and voluntary lunch intake, VG n = 18). [2]The percent change is calculated as the difference from HG and VG to the WWB.

Figures

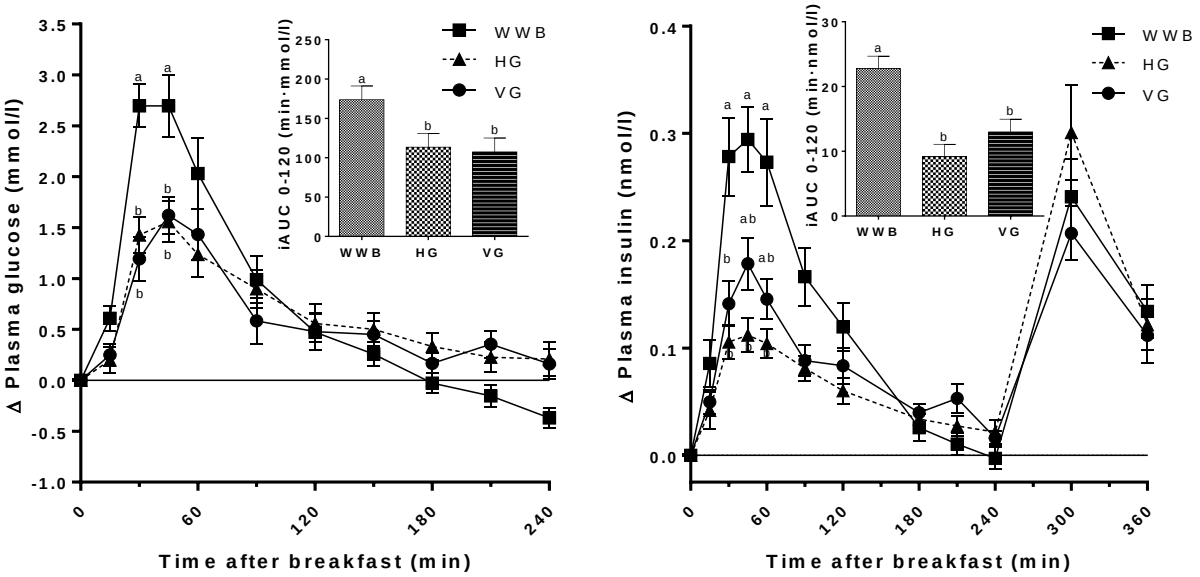

Figure 1: Mean incremental changes (Δ) and iAUC 0-120 min in plasma glucose and insulin (mean and LSMs ± SEM, respectively), n = 19, (VG n = 18).

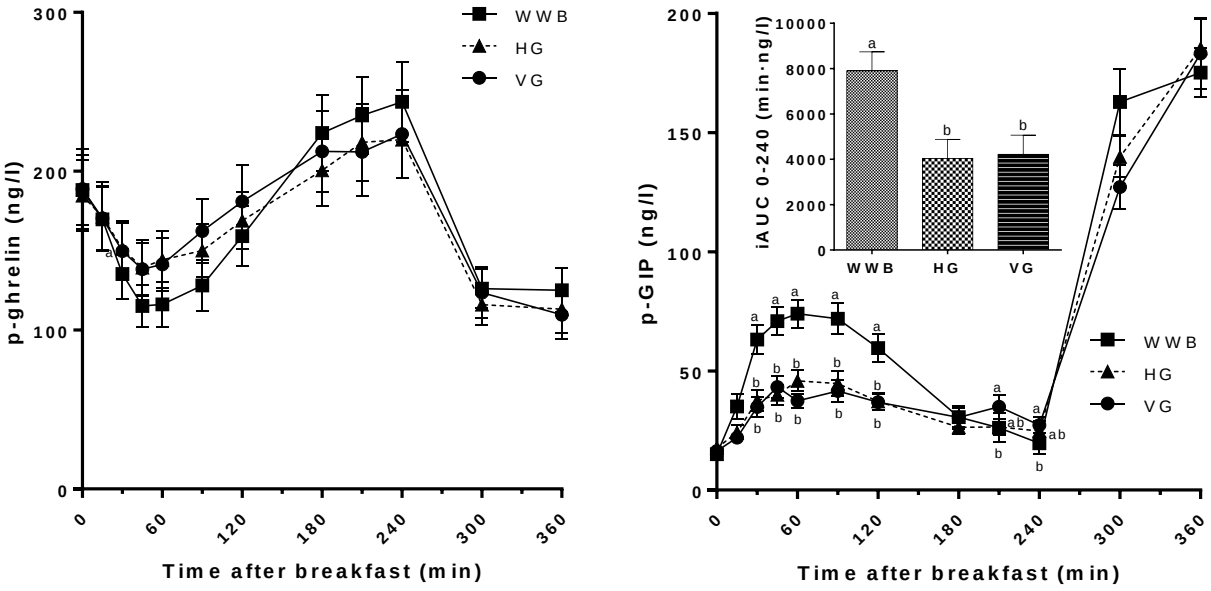

Figure 2: Postprandial change in ghrelin and GIP (mean ± SEM) and iAUC 0-240 min for GIP (LSMs ± SEM), n = 19, (VG n = 18).

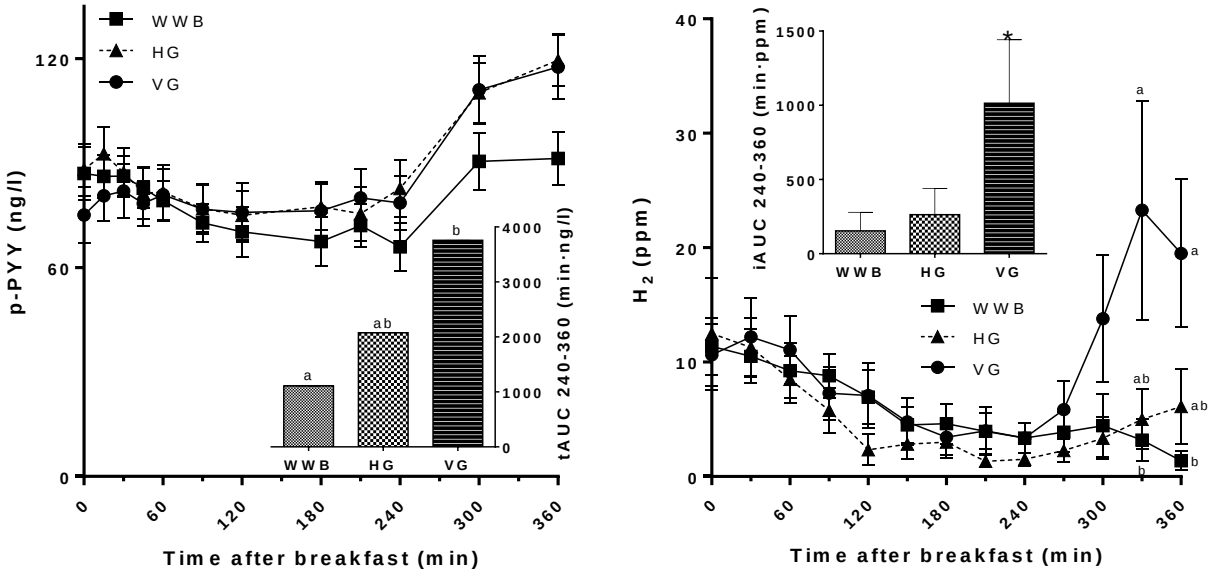

Figure 3: Postprandial changes and i- or tAUC 240-360 min for PYY and breath H_2 (mean and LSMs ± SEM, respectively). PYY n = 18, (VG n = 17), breath H_2 WWB n = 16, HG n = 18, VG n = 14, * p = 0.058.

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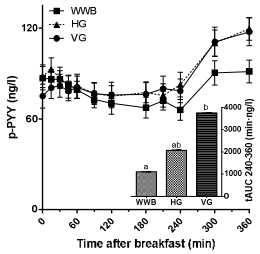
Table of contents entry



A breakfast giving low and sustained glycaemia results in beneficial effects on appetite, both acute and after a subsequent *ad lib* meal.