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ORIGINAL CONTRIBUTION

Enrichment of bread with beta-glucans or resistant starch induces similar glucose, insulin and appetite hormone responses in healthy adults

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Abstract

Purpose β-Glucans (βG) and resistant starch (RS) are known for their effects on the improvement of glucose tolerance and enhancement of insulin sensitivity. Enrichment of bread with βG or RS was performed to examine potential postprandial benefits regarding gastrointestinal hormone responses.

Methods Ten healthy normoglycaemic adults participated in the study and were provided with either a glucose solution (reference food, GS) or bread enriched with β-glucans (βGB) (3.6 g/30 g available CHO) or bread enriched with resistant starch (RSB) (15% of total starch), with 1-week intervals in amounts that yielded 50 g of available carbohydrates. Venous blood samples were collected before consumption and at 15, 30, 45, 60, 90, 120 and 180 min postprandially. Glucose, insulin, ghrelin, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) responses as well as glycaemic index (GI) and subjective appetite ratings were evaluated.

Results Ingestion of βGB and RSB elicited lower incremental area under the curve (iAUC) for glycaemic response compared to GS ($P < 0.05$). Both breads demonstrated a low GI (βGB: 48, RSB: 40). There were no significant differences in insulin response, ghrelin, GLP-1 or PYY between the two breads. A significantly lower desire to eat and higher fullness were detected 15 min after βGB and RSB consumption and until 180 min ($P < 0.05$ compared to GS).

Conclusion Enrichment of bread with either βG or RS produced a low GI product but the two breads were not significantly different in relation to insulin, ghrelin, GLP-1 and PYY responses. The development of bread products which cause improved metabolic effects is of great importance for the promotion of public health.

Keywords Bread · β-glucans · Resistant starch · Postprandial hormone responses · Glycaemic index · Appetite

Introduction

Postprandial glycaemia is found to be directly associated with chronic non-communicable diseases, such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), even several types of cancer, according to epidemiological evidence [1–3]. Carbohydrates, which represent the highest percentage of energy intake in humans' diet, typically accounting for 45–70%, mainly determine the postprandial glucose levels [4].

Bread is a staple food and a primary source of carbohydrates in most European countries. Specifically, the average bread consumption in Europe is about 170 g per day per capita [5]. Among starchy foods, white bread prepared with wheat flour is the most frequently consumed cereal product, due to its palatability and great sensory

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characteristics [6]. Nonetheless, its porous matrix and high gelatinized starch content, classify it as a high glycaemic index (GI) food [7].

Lowering the GI of bread is of scientific interest considering the potential benefits of a low glycaemic diet on the risk and the management of T2DM and heart disease [8]. Among successful strategies, are included the addition of soluble dietary fibers such as β-glucans (βG) concentrates originating from oats or barley or the addition of resistant starch (RS) [9, 10].

Consumption of meals rich in βG is associated with the improved postprandial metabolic profile. The potential mechanism that can explain this beneficial effect seems to be their ability to increase the viscosity of the gut digesta, and thus the delay of the carbohydrate absorption from the gut [11]. This health claim was approved by European Food Safety Authority (EFSA) in 2011. Particularly, a food that contains at least 4 g of βG from oats or barley for each 30 g of available carbohydrates in a quantified portion as part of the meal can ameliorate the postprandial glucose rise [12, 13]. In fact, it has been shown that the natural content of βG in oats and barley flour is insufficient to meet the requirements of the health claim, in comparison with the addition of high-βG containing flour in bread, which seems to be able to reduce the glucose response [9].

Despite viscous fibers, improved postprandial glycaemia is also documented by non-viscous fibers, such as RS [14]. RS is defined as a starch fraction, or the products of its degradation, which is not digested in the upper gastrointestinal tract but fermented by the colonic microbiota [15–17]. There is increasing interest from the food industry about the development of RS containing products, not only due to its beneficial effects on glucose and lipid metabolism but also because of its sensory characteristics. More specifically, RS has white color, neutral flavor and small particle size, characteristics that may offer to fortified products, palatability and relevant tolerance in the usual gastrointestinal symptoms that follow the fiber consumption [18]. In parallel to the health claim for βG, EFSA, respectively, accepted as health claim that replacing digestible starches with RS in a meal contributes to a reduction in the postprandial blood glucose rise. The final content of RS must be at least 14% of the total starch in the meal [19].

The aim of the present study was to compare the postprandial metabolic effects of two different functional breads enriched either with βG (βGB) or resistant starch (RSB) in amounts suggested by the EFSA health claims. Glycaemic and insulinaemic responses, as well as ghrelin, GLP-1 and PYY responses were examined and were accompanied by estimation of subjective appetite ratings. Sensory characteristics of the enriched products were also evaluated.

Materials and methods

Subjects

A randomized, single-blind, crossover-designed trial was conducted at the 1st Department of Propaedeutic Internal Medicine, Laiko General Hospital and the Laboratory of Experimental Surgery and Surgery Research, Athens University Medical School, in collaboration with Laboratory of Chemistry, Biochemistry and Physical Chemistry of Foods. Ten apparently healthy subjects (five men and five women) aged between 23 and 35 years (mean 27 years; SD 3.9) with a normal body mass index (mean 24.5 kg m^{-2}; SD 2.8) were recruited for the study by means of poster and electronic advertisements. They had a stable weight for at least 3 months before enrollment and normal exercise, eating and drinking habits, as ascertained by nutritional assessment interview at screening. According to ISO 26642 for the determination of the GI ten healthy subjects are required.

Exclusion criteria were an age younger than 18 years old or older than 60 years old, pregnancy and lactation, chronic medical illness (diabetes mellitus, cardiovascular disease, chronic liver, kidney or untreated thyroid disease), use of nutritional supplements which could interfere with the results, a history of drug and/or alcohol abuse or psychiatric disease prohibiting adherence to the protocol. Eligible subjects were enrolled in the study after being informed in detail about its nature and all procedures and giving their written consent for participation. The protocols were reviewed and approved by both the Institutional Review Board/Ethics Committee of Laiko Hospital and the Harokopio University of Athens.

Study design

The study was designed as an acute, randomized controlled cross-over study with two types of bread and a solution containing 50 g of glucose, as the reference food. After meeting inclusion criteria, subjects were invited to visit the Diabetes Laboratory of the 1st Department of Propaedeutic Internal Medicine, Laiko General Hospital, four times in total, at 9:00 a.m. after a 12-h overnight fast. These four testing sessions, which were separated by intervals of one week, included consumption of the following: wheat bread enriched with β-glucans (βGB), wheat bread enriched with resistant starch (RSB) in random order and the glucose solution (GS), which was consumed twice, at the first and the last session respectively, according to ISO 26642.

Volunteers who participated in the study were advised not to drink alcohol or perform exercise on the day before

each trial, as well as to maintain the same dietary habits and physical activity level until the completion of the study. Bodyweight, body fat, height and blood pressure measurements were performed at the first session, at the beginning of the study. Particularly, body weight was measured with light clothing on a scale (TANITA WB-110MA, Japan) and body fat by bioelectrical impedance analysis (Tanita BC-418, Tokyo, Japan). Height was measured using a stadiometer (Seca Mode 220, Hamburg, Germany) with subjects not wearing shoes, their shoulders in a relaxed position and their arms hanging freely. Waist circumference was determined at the midpoint between the lower margin of the least palpable rib and the top of the iliac crest in a standing position at the end of gentle expiration. Hip circumference measurement was taken around the widest portion of the buttocks. Energy intake and expenditure data were obtained by a dietician with the use of a 24-h dietary recall and a physical activity questionnaire respectively, at each one of the four sessions.

After 10 min of rest, an intravenous catheter was placed in a forearm vein and baseline samples were drawn (time 0). Then, a test meal of either a type of bread in an amount corresponding to 50 g of available carbohydrates or a solution containing 50 g of glucose diluted in 250 mL of water was served and subjects were advised to consume it within 10 min. Blood samples were collected before food ingestion and 15, 30, 45, 60, 90, 120 and 180 min postprandially. At the end of the intervention, the catheter was removed and the participants were evaluated for wellbeing prior to leaving Laiko Hospital.

Test breads

Meals designed for the study included a wheat bread enriched with β-glucans (βGB) and a wheat bread enriched with resistant starch (RSB), high amylose waxy maize starch. Breads were prepared by adding β-glucans or RS in the dough mixture. The dough was rested for almost 3 min at room temperature, divided into 900 g portions, placed in baking tins and then put in proofing chamber for 60 min at 35 °C and relative humidity 80%. Tins with bread dough were baked in horizontal continuous flow belt oven with temperature regime between 205 and 275 °C for 28–30 min.

The nutritional composition of the two tested breads is presented in Table 1. Nitrogen (protein: Nx6.25) was measured by Kjeldahl (ISO 1871) and fat by Soxhlet procedures. Total dietary fibers were determined by the AOAC method 991.43, βG by the AOAC method 995.16 and RS by the AOAC method 2002.02 using a Megazyme Kit (Megazyme International Ireland Ltd, Wicklow, Ireland). On Table 1, total starch represents the total amount of starch including the RS.

Blood analysis

Blood samples were collected in pre-cooled vacutainers with K_3EDTA as anticoagulant and centrifuged immediately (3000 rpm for 10 min at 4 °C) for plasma separation. For serum, blood was collected in plain vacutainers, allowed to clot at room temperature for 30 min and then centrifuged (3000 rpm for 10 min at 4 °C). After isolation, plasma and serum were stored at − 80 °C until analysis.

For total ghrelin determination, plasma was pretreated according to the procedure described in a previous study [20]. Ghrelin at 0, 30, 60, 120 and 180 min was assayed by a sandwich ELISA method on a microtiter plate using a commercially available human ghrelin kit [Human Ghrelin (Total) ELISA kit; Millipore]. Insulin was similarly detected by a sandwich ELISA method using a commercially available human insulin kit (Human Insulin ELISA kit; Millipore) at 0, 15, 30, 45, 60, 90, 120 and 180 min, in serum. Total GLP-1 and PYY at 0, 15, 30, 60, 90, 120 and 180 min were also assayed by a sandwich ELISA method using a commercially available human GLP-1 kit (Human Total Glucagon-Like Peptide-1 kit; Millipore) and a human PYY kit [Human PYY (Total) ELISA kit; Millipore], respectively. Glucose concentration at 0, 15, 30, 45, 60, 90, 120 and 180 min was measured in plasma by YSI 2300 STAT Plus Glucose Lactate Analyzer. At the beginning of the study, basal biochemical measurements including the total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triacylglycerols (TAG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyl transferase (γ-GT), urea, creatinine, uric acid

Table 1 Nutritional composition of the two tested types of bread, each providing 50 g of available carbohydrates

Meals	Serving size (g)	Energy content (Kcal)	Available CHO (g)	Fat (g)	Protein (g)	Total dietary fibers (g)	β-glucans (g)	Resistant starch (g)	Total starch (g)
βGB	166	410	50	8.3	27.2	13.8	6.0	–	47.0
RSB	166	393	50	7.6	21.8	19.1	–	8.8	59.0

Data are presented per serving. Total dietary fibers include the amounts of β-glucans and resistant starch in each bread respectively

βGB wheat bread enriched with β-glucans, *RSB* wheat bread enriched with resistant starch

and total proteins were performed in serum, automatically, by an automated biochemical analyzer (Medilyzer), using commercially available diagnostic kits.

Subjective appetite

Subject's appetite ratings, specifically, hunger, fullness and desire to eat after the consumption of the four test meals were assessed at the times 0, 15, 30, 45, 60, 90, 120 and 180 with the use of visual analogue scales (VAS). Three main questions were included, "How hungry do you feel", "How full do you feel" and "How great is your desire to eat". Subjects were asked to rate these three parameters on a 10-cm line scale ranging from 0 ("not at all") to 10 ("extremely"), with labels at the extremities indicating the most positive and negative ratings. Subjects were not allowed to discuss their ratings with each other throughout the sessions.

Sensory evaluation

The subjects who participated in the study were asked to evaluate the sensory characteristics of the two types of bread (βGB, RSB). Palatability of the breads in terms of texture, color, aroma, taste and overall acceptability on a 9-point hedonic scale (1 = extremely dislike, 5 = neither like nor dislike, 9 = extremely like) were evaluated. The hedonic scale is a standard tool for measuring food acceptability, that is, how much a consumer likes or dislikes a product.

Statistical analysis

Descriptive statistics are presented as mean ± SD and the results as mean ± SEM. The incremental area under the curve (iAUC) was calculated for glycaemic and insulinaemic responses, ghrelin, GLP-1 and PYY applying the trapezoidal rule. Accordingly, iAUCs for VAS were estimated. The GI was determined as the mean individual ratios resulting from iAUC for glucose over a period of 120 min after ingestion of βGB and RSB relative to that after ingestion of the glucose solution. ANOVA for repeated measures, followed by the post hoc Bonferroni's test, was used to identify significant differences regarding postprandial concentrations of plasma glucose, subjective appetite ratings and the respective iAUCs between treatments. A paired sample Students *t*-test was used to compare insulin, ghrelin, GLP-1 and PYY postprandial levels at specific time points as well as iAUCs between βGB and RSB. Students t-test was also performed to estimate the differences for the sensory properties. $P < 0.05$ was considered statistically significant. The SPSS 21.0 statistical software package was used for the analyses.

Table 2 Anthropometric and biochemical characteristics of the subjects

Characteristic	Value
N	10
Sex (male/female)	5/5
Age (years)	27.0 ± 3.9
BMI (kg/m^2)	24.5 ± 2.8
WC (cm)	82.4 ± 8.6
Body fat (%)	23.1 ± 9.3
SBP (mmHg)	115.7 ± 10.3
DBP (mmHg)	71.8 ± 7.6
Fasting plasma glucose (mg/dL)	92.5 ± 7.5
Cholesterol (mg/dL)	185.5 ± 36.6
HDL-C (mg/dL)	65.9 ± 10.3
LDL-C (mg/dL)	102.5 ± 38.9
Triacylglycerols (mg/dL)	79.5 ± 47.8
AST (U/L)	20.7 ± 6.6
ALT (U/L)	22.5 ± 5.4
γ-GT (U/L)	27.1 ± 6.6
Urea (mg/dL)	37.8 ± 6.7
Creatinine (mg/dL)	0.9 ± 0.1
Uric acid (mg/dL)	4.8 ± 1.2
Total proteins (mg/dL)	7.2 ± 0.5

Values are expressed as mean ± SD

BMI body mass index, *WC* waist circumference, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *γ-GT* γ-glutamyl transferase

Results

The anthropometric and basal biochemical characteristics of the 10 subjects who completed successfully the four sessions of the study are presented on Table 2.

The GI of the two types of bread was found to be lower than 55 i.e. for βGB, 48 ± 10 and for RSB 40 ± 8. The mean individual ratios of iAUC resulting from iAUC over a period of 120 min for each of the test meals compared with that of the reference meal of the glucose solution were used for the calculation of GI.

The glycaemic response to βGB, RSB and reference meal (GS) was determined over a 3 h period after the consumption of each test meal and is presented in Fig. 1a. Both breads ameliorated postprandial glycaemic response. The consumption of the reference meal caused the highest iAUC, i.e. 2818 ± 498 mg dL^{-1} × 180 min. iAUCs for RSB and βGB are presented in Table 3. The difference was statistically significant for the two breads compared to GS ($P < 0.05$). Mean peak glucose concentration was higher after GS followed by RSB and βGB (142.2 ± 7.6 compared to 113.8 ± 3.9 and 115.0 ± 4.3 mg/dL, respectively, $P < 0.05$). No statistically

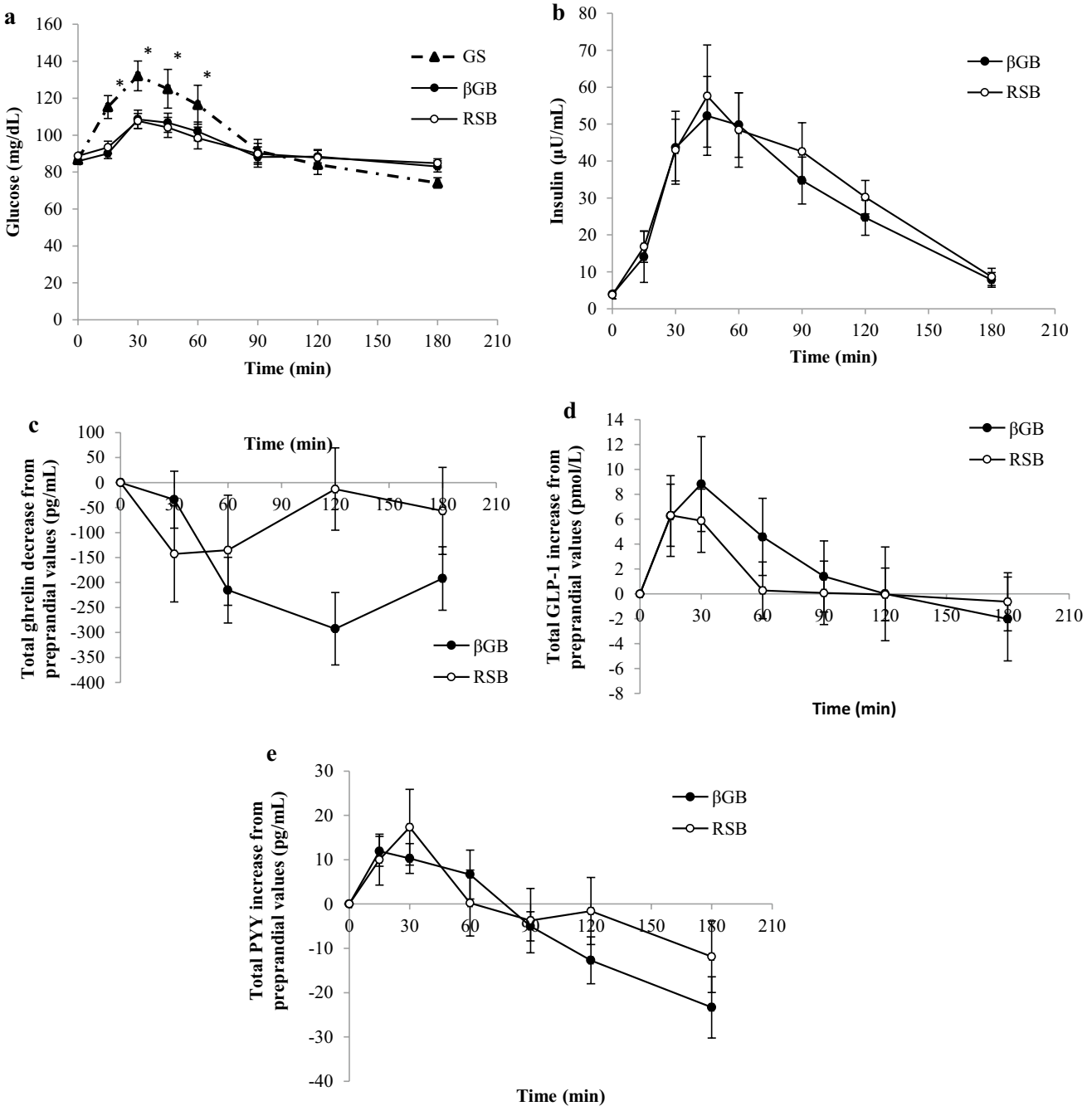

Fig. 1 Responses of glucose (**a**), insulin (**b**), ghrelin (**c**), GLP-1 (**d**) and PYY (**e**) for 180 min after the consumption of the two breads and the solution of glucose. Values are expressed as mean ± SEM ($N = 10$). *$P < 0.05$ compared to GS

Table 3 Incremental area under the curve (iAUC) for glucose, insulin, ghrelin, GLP-1 and PYY after consumption of the two types of bread

iAUC	βGB	RSB	*P*
Glucose (mg $dL^{-1} \times 180$ min)	1454 ± 353	1168 ± 320	0.333
Insulin (µU $mL^{-1} \times 180$ min)	3747 ± 716	4156 ± 680	0.507
Ghrelin (pg $mL^{-1} \times 180$ min)	− 35,430 ± 9462	− 28,983 ± 7455	0.449
GLP-1 (pmol $mL^{-1} \times 180$ min)	946 ± 289	604 ± 258	0.190
PYY (pg $mL^{-1} \times 180$ min)	811 ± 235	1533 ± 596	0.283

Values are expressed as mean ± SEM

significant difference was noticed between the two types of bread.

Insulin response to the two different types of bread is illustrated in Fig. 1b. The iAUC did not differ between the two types of bread ($P=0.507$). The peak insulin value was observed at 45 min postprandially after the consumption of βGB and RSB and no significant difference was detected between them (52.2 ± 10.7 and 57.6 ± 13.8 μU/mL, respectively, $P=0.668$).

Total ghrelin response expressed as a decrease from preprandial values is presented in Fig. 1c. The iAUC did not differ between the two types of bread ($P=0.449$). Moreover, both breads caused similar ghrelin suppression at the individual time points without any statistically significant differences. However, βGB caused the maximum suppression at time point 120 min while RSB at 30 min. At the time point 120 min there was a marginally statistically significant difference ($P=0.1$) with the βGB leading to the larger decline of ghrelin secretion compared to RSB (-292.52 ± 72.52 and -130.11 ± 82.39 pg/mL, respectively).

Plasma GLP-1 and PYY concentrations expressed as an increase from preprandial values are illustrated in Fig. 1d, e respectively. There were no statistically significant differences between the iAUC of the two breads neither for GLP-1 nor for PYY ($P=0.190$, $P=0.283$, respectively). The plasma GLP-1 concentrations were higher during the first 60 min after the consumption of βGB than RSB. The difference was marginally significant ($P=0.1$ at the time point 60 min).

Figure 2 shows the subjective appetite rating differences from preprandial values over the 180 min after the consumption of the test breads. Perceived appetite for hunger, fullness and desire to eat was similar in response to the two breads ($P>0.5$). iAUC values did not indicate any significant

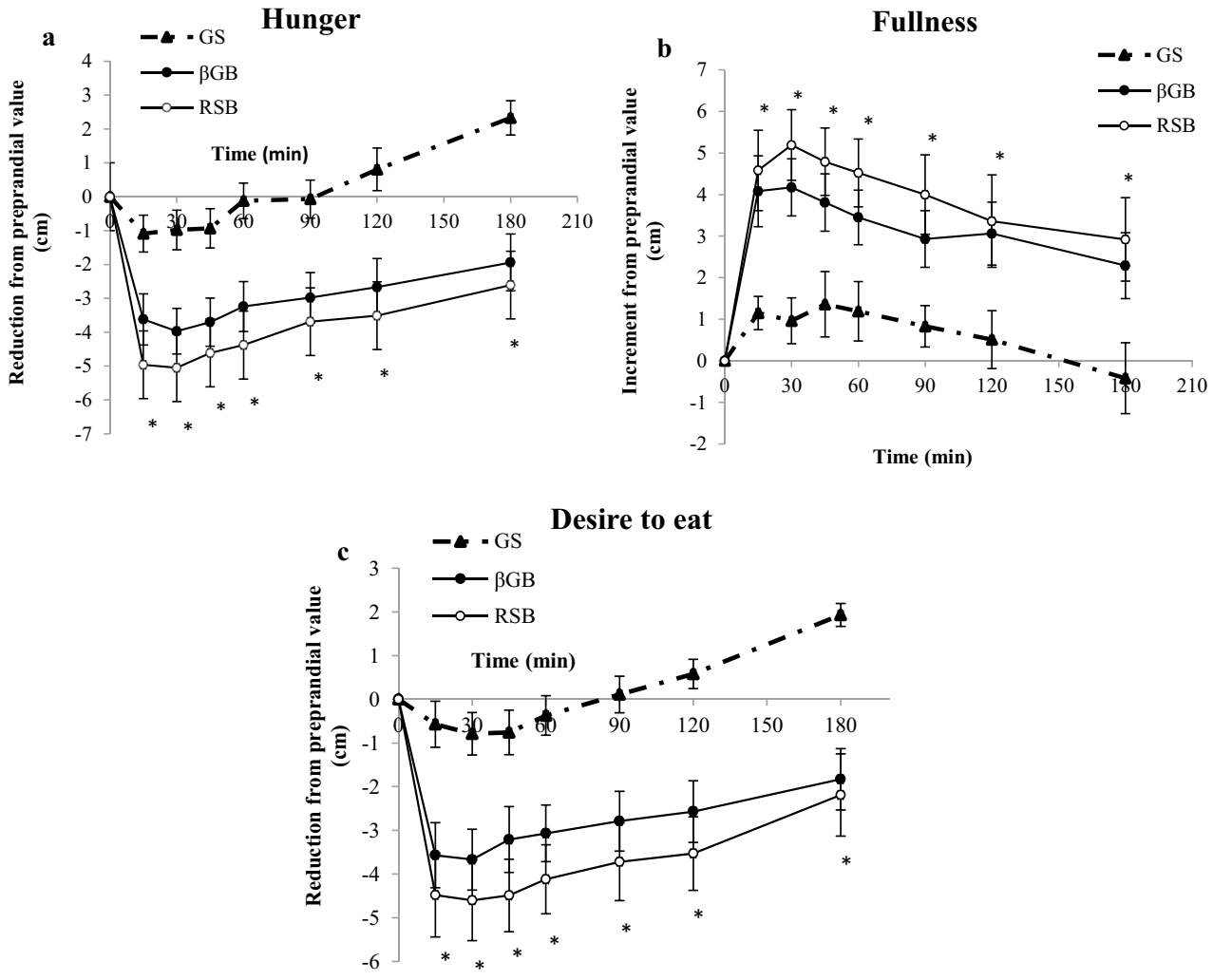

Fig. 2 Mean subjective appetite rating differences from preprandial values of hunger (**a**) fullness (**b**) and desire to eat (**c**) after the consumption of the two breads and the solution of glucose. Values are expressed as mean ± SEM ($N=10$). *$P<0.05$ compared to GS

Table 4 Incremental area under the curve (iAUC) for subjective appetite ratings (hunger, fullness and desire to eat) after consumption of the two types of bread and the reference food (glucose solution)

iAUC	GS	βGB	RSB	P_1	P_2
Hunger (cm × 180 min)	− 100 ± 38	− 530 ± 120*	− 675 ± 152*	0.005	0.004
Fullness (cm × 180 min)	185 ± 80	567 ± 112*	701 ± 150*	0.001	0.002
Desire to eat (cm × 180 min)	− 95 ± 38	− 486 ± 114*	− 622 ± 140*	0.009	0.009

Values are expressed as mean ± SEM

*$P_1 < 0.05$ between βGB and GS, *$P_2 < 0.05$ between RSB and GS

Table 5 Sensory attributes of the two breads

Sensory attributes	βGB	RSB
Texture	7.1 ± 0.3	7.1 ± 0.3
Color	6.8 ± 0.4	7.4 ± 0.2
Aroma	6.6 ± 0.34	6.5 ± 0.2
Taste	6.3 ± 0.6	6.7 ± 0.6
General acceptance	6.5 ± 0.5	6.9 ± 0.5

Values are expressed as mean ± SEM

differences between the test breads (Table 4). However, significantly lower hunger, lower desire to eat and higher fullness were observed at all time points, after the consumption of the two breads in comparison to GS ($P < 0.05$).

Regarding sensory evaluation, both breads scored similarly and achieved high overall acceptance scores (Table 5). More specifically, βGB had an overall score of 6.5 ± 0.5 and RSB of 6.9 ± 0.5 ($P = 0.1$) which showed no statistical significance, but a trend that RSB tends to have better palatability than βGB.

Discussion

A rapid increase in the incidence of chronic metabolic disorders [21, 22] has created an urgent need of finding strategies that aim to their prevention and treatment. In conjunction with growing scientific evidence that associate mild postprandial glycaemia with beneficial metabolic outcomes [23–25], the development of low GI products arises as a potential useful practice. Techniques that seem to have promising results are the enrichment of foods with dietary fibers either soluble or insoluble.

White bread is reported as a high GI food (75 ± 2) [26]. In the present study, both breads are found to have a low GI, below 55 as it was expected since the content of β-glucans and resistant starch were close or exactly (respectively) the ones suggested by EFSA. The determination of GI was accomplished according to ISO 26642 and glucose solution was selected as a reference food and was tested twice, so the classification could be made by using the GI scale.

More specifically, the GI of βGB was found to be 48 ± 10, with glucose as a reference food. βGB lowered the postprandial glycaemic response by 48%. These findings are in accordance with previous clinical trials, which have shown that enrichment of breads with βG results to milder glycaemic response [27–31]. The mechanism of action that explains the result is well established. βG is very high molecular weight polysaccharides that exhibit high viscosities at low concentrations. Consequently, the enrichment of foods with this kind of fiber slows the mixing of the meal with the digestive enzymes and delays the gastric emptying, and thus retards the absorption of glucose [12, 32]. The dose of βG that can achieve the attenuation of postprandial glucose is 4 g for 30–80 g of available carbohydrates [32]. In the present study, the βGB contains 3.6 g per 30 g, a sufficient amount that can explain the lower GI of the bread. Recent study by Ekström et al. reported that even the amount of 1.9 g of βG per 30 g of available carbohydrates gave significant lowering in postprandial glucose levels (GI = 64 ± 5) [31].

Second aim of the present clinical trial was the evaluation of the satiety caused by the two different fortified breads. To this purpose, except for the examination of visual analogue scales (VAS), gastrointestinal hormones such as ghrelin, PYY and GLP-1 were measured.

Ghrelin is a peripheral hormone, that is synthesized in the gastrointestinal tract, especially in the fundus of the stomach, and its concentration is directly related to appetite ratings. In normal-weight subjects, plasma ghrelin decreases after the consumption of a meal. On the other hand, PYY, a hormone derived mainly from the colon, is considered to have potent anorexigenic properties [33]. Another peptide that acts as a satiety signal and increases postprandially is the GLP-1. This hormone is produced by the L cells of the distal small intestine in response to a nutrient load and its effect on the modulation of appetite is orchestrated by a complex brain-gut relationship [34].

The relationship of βG and the postprandial hormonal concentrations is not fully clarified. Research from Vitaglione et al. suggests that βG enriched bread is able to modulate appetite ratings through the significant alteration of ghrelin and PYY levels [28]. Similar outcomes were observed even

if the βG were incorporated in other meals, such as biscuits, rice or juices [35–38]. As previously referred, βG are a very viscous fiber, a characteristic that gives to the enriched meal prolonged transit time and the absorption rate of nutrients. The prolonged presence of nutrients in the GI tract raises the possibility of interaction between nutrients and the intestinal mucosa to stimulate the release of peptides involved in appetite regulation. On the other hand, Juntunen et al. [39] showed that the GLP-1 response did not differ between bread with βG and white bread. Another more recent clinical trial, also reported that consumption of barley bars containing 1.2 g of barley βG did not change appetite and energy intake when compared with oat bars containing 0.3 g of oat βG [40].

The iAUCs for hunger, fullness and desire to eat were statistically significant different between the bread and the reference food, however, no significant difference was observed between the two breads. The use of VAS to measure variability of appetite, despite of being a subjective method, has reproducibility and seems to be connected with the levels of satiety hormones [41, 42].

With regard to RS, thorough research has been conducted, in both humans and animal models, for its effects in postprandial glycaemic response. Particularly, it lowers glycaemia when it replaces the available carbohydrates portion of meal [15]. In accordance with these findings, in our study RSB had an advantage over the glucose solution, as it lowered the postprandial glycaemic response by 59%. A plethora of studies has examined RS role in improving a variety of metabolic features. Previous clinical trials conducted in healthy subjects reported great reductions in postprandial glucose response after the consumption of RS enriched breads or crackers in comparison with control of white wheat bread [43–49]. In some cases, there was even found an improvement in insulin sensitivity, when RS represented the 10.8% to 12.3% dry matter of bread, in comparison with approximately 2% in the reference food (white wheat bread) [48, 49].

Moreover, a few studies in which healthy participants consumed rice or plantains containing a high amount of RS have shown a correlation between RS content and milder glycaemic response [50–53]. Attempts have been made to understand how RS influences glycaemic control. Its beneficial effects may be achieved through colonic production of short-chain fatty acids (SCFAs), then subsequent absorption, by exerting anti-lipolytic activity and affecting the activity of gut hormones, such as GLP-1, which concentrations increase following the consumption of RS-containing foods and stimulate insulin secretion [54]. In our study βGB caused higher ghrelin suppression than RSB however, a significant difference in iAUC was not observed probably due to the small number of individuals. The two breads caused similar GLP-1 and PYY rise. A higher increase in GLP-1 was noticed after the consumption of βGB during the first 60 min of the postprandial state.

The GI of RSB was measured 40 ± 8, with glucose as a reference food. The dose of RS that should be used to achieve a milder postprandial glucose is 14% of the total starch [20]. In the present study, the RSB contains about 15%, an amount that can explain the lower GI of the bread.

Consumption of RSB leads to decrease of hunger and desire for the next meal and increase in fullness. Similar results have been found in other clinical studies in which participants consumed breads enriched with RS and the results were compared with control bread with low concentration in RS [44, 55–57]. An intervention study comparing a high RS with a control diet have shown a reduction in hunger and increase in subjects' satiety after the consumption of the first diet [58].

Regarding GLP-1 and PYY there has been reported in previous studies a correlation between consumption of bread rich in RS and an increase in these hormones' postprandial concentrations in plasma [44, 48, 49, 59]. Intervention studies have shown beneficial effects in GLP-1 or PYY levels of a high RS compared to a control diet [60, 61]. It is also noteworthy that in a study in a rodent population, RS appeared to stimulate the secretion of GLP-1 and PYY from the intestine [62].

Between the two breads no statistically significant differences in the secretion of gut hormones were observed. However, there was a trend of a larger decline of ghrelin secretion and a higher concentration of GLP-1 after the consumption of βGB compared with RSB at time points 120 min and 60 min, respectively. This can be explained by taking into consideration that RS, as a non- viscous fibre, does not delay gastric emptying in a similar way to that observed with viscous fibers [15, 40, 63].

The results indicated a great effect of both breads enriched with either βG or RS in postprandial glycaemia compared with the reference food. There was also statistical significant difference in the VAS analogue scales for the two breads compared to the reference food. There were found no statistically significant differences in glucose or insulin responses after the ingestion of βGB and RSB. The two breads showed similar sensory characteristics and had great overall acceptability from the participants. According to our knowledge, this is the first study to compare the effects of beta-glucans and RS when supplemented in amounts suggested by EFSA, on bread postprandial glycemic and hormonal responses.

In conclusion, the addition of βG or RS to wheat bread can lead to a low GI food which may induce satiety. The replacement of white wheat bread with any of these two types of bread can be a positive nutritional alteration which can provide metabolic benefits for the prevention and management of chronic diseases such as diabetes and obesity.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard All human studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study.

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