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An Enriched, Cereal-Based Bread Affects Appetite Ratings and Glycemic, Insulinemic, and Gastrointestinal Hormone Responses in Healthy Adults in a Randomized, Controlled Trial[1,2]

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Abstract

Background: Bread can contribute to the regulation of appetite.

Objective: The objective of this study was to investigate the appetite ratings and postprandial glucose, insulin, and gastrointestinal hormone responses related to hunger and satiety after the intake of a cereal-based bread.

Methods: A randomized, controlled crossover trial was conducted in 30 healthy adults (17 men and 13 women) aged 19–32 y with body mass index of 19.2–28.5. Each volunteer consumed the cereal-based bread and the control bread 2 times, with a 1-wk wash-out period, over a total of 4 sessions. The cereal-based bread contained a variety of cereal flours (wheat, oat, and spelt) and consisted of 22% dried fruits (figs, apricots, raisins, and prunes). It was also enriched with both fiber (7% from wheat cross-linked maltodextrins and pea) and protein (10–11% from wheat gluten and hydrolyzed wheat proteins). The control bread consisted of white bread with margarine and jam to control for energy density, fat, and sugar content. We measured appetite ratings using standardized visual analogue scales and glucose, insulin, and gastrointestinal hormone responses over a postprandial time of 4 h after the ingestion of each bread. Linear mixed-effects models were used to compare the areas under the curve (AUCs) for different variables.

Results: Consuming the cereal-based bread decreased prospective consumption more than consumption of the control bread (-5.3 ± 0.6 m · min and -4.4 ± 0.6 m · min, respectively; $P = 0.02$) and increased satiety more (6.2 ± 0.7 m · min and 5.2 ± 0.6 m · min, respectively; $P = 0.04$), although subsequent ad libitum energy intake 4 h later did not differ. Postprandial blood glucose, insulin, ghrelin, glucagon-like peptide 1 and gastric inhibitory polypeptide AUCs were lower after the ingestion of the cereal-based bread, whereas the pancreatic polypeptide AUC was higher than with the control bread ($P < 0.05$).

Conclusions: Consumption of the cereal-based bread contributed to appetite control by reducing hunger and enhancing satiety. In addition, consumption of this bread improved glycemic, insulinemic, and gastrointestinal hormone responses in healthy adults. This trial was registered at clinicaltrials.gov as NCT02090049. *J Nutr* 2015;145:231–8.

Keywords: appetite, bread, dietary fiber, gastrointestinal hormones, glycemia, insulinemia, satiety

Introduction

The condition of being overweight or obese represents one of the most important public health issues worldwide; excess body weight is currently the sixth most important risk factor contributing to the overall burden of disease (1). It is well known that obesity is a risk factor for metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease (2); therefore, there is a great need to find preventive strategies for reducing weight gain and, thus, the incidence of metabolic diseases. The regulation of appetite is a vital component of general physical and psychological health. Food itself plays a role in maintaining energy balance, providing pleasure and sensory satisfaction. The "not eating breakfast" issue is a worldwide phenomenon because of a lack of time in the morning; therefore, an increasing number of people are looking for a practical breakfast solution that is quick and easy. The food industry can offer foods that match the

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population's current needs, providing balanced and convenient breakfast products that *1*) help consumers improve their appetite control to avoid weight gain, and *2*) allow better nutrient intake. The European Food Safety Authority recently provided a guidance document with scientific requirements for health claims related to appetite ratings and blood glucose concentrations (3).

Postprandial glycemia, insulin secretion, and hormones released by the gastrointestinal tract before or during nutrient ingestion play key roles in maintaining appetite regulation. These hormones include the orexigenic ghrelin and the anorexigenic and metabolic hormones glucagon-like peptide 1 (GLP-1)[9], oxyntomodulin, peptide YY (PYY), pancreatic polypeptide (PP), and cholecystokinin (4, 5). In addition, GLP-1 and gastric inhibitory polypeptide (GIP) are involved in the regulation of insulin secretion (6). For these reasons, these hormones have been investigated for the treatment of obesity.

High fiber intake has been associated with a lower BMI (7–9), as well as a lower risk of the progression of type 2 diabetes mellitus, cardiovascular diseases, and metabolic syndrome (10, 11). In recent years, dietary fiber has been recognized as playing a potential role in appetite regulation (12); however, the food matrix may modulate the effect of each individual ingredient. The effect of bread intake and the type of bread components responsible for the modulation of food intake are poorly understood (13). The aims of this study were to *1*) evaluate the appetite ratings of a cereal-based bread with a high content of fiber and protein and *2*) determine its capacity to modulate postprandial glucose, insulin, and the plasma concentrations of a subset of gastrointestinal hormones involved in appetite control and insulin secretion in healthy adults.

Methods

Study design. A prospective randomized, controlled crossover single-blind study was employed to evaluate a new ready-to-eat cereal-based bread provided as a breakfast, "Puravita Breakfast," compared with a control bread plus margarine and jam to adjust for energy, fats, and sugars. The experimental bread was a cereal-based bread containing a well-balanced variety of cereal flours (wheat, oat, and spelt) and 22% dried fruits (figs, apricots, raisins, and prunes), and enriched with fiber (7% added fiber consisting of wheat cross-linked maltodextrins and pea fiber, resulting in 6.0% soluble and 4.1% insoluble fiber) and protein (10–11% added from wheat gluten and hydrolyzed wheat proteins), with no added sugar. This bread was manufactured by Puratos. Because we aimed to measure the satiety effect linked to the substitution of carbohydrates (starch) with fiber and protein, we matched the composition of the control bread with fat and sugar. To control for energy density, fat, and sugar levels, the control bread consisted of commercially available sliced white bread (made with wheat flour, water, yeast, sugar, dextrose, salt, and dough conditioners; 85 g) to which jam (made with 16% strawberries, 16% cherries, 9% raspberries, and 9% red currants; 10 g) and margarine (made with rapeseed, palm, sunflower, and soy oils; 2 g) were added. The nutritional composition of each bread is specified in **Table 1**. Each morning, the 2 types of breads were thawed at room temperature, and the margarine and jam were weighed and prepared exclusively for the control bread.

Subject selection and allocation. A diagram based on the Consolidated Standards Of Reporting Trials for the selection, allocation, and crossover random assignment of the participants who were involved in the study is shown in **Figure 1**. A total of 55 healthy participants (aged 18–35 y) were initially selected in October 2012 from a group of students pursuing a degree in human nutrition and dietetics at the University of Granada. To avoid the risk of reaching false conclusions, psychometric validations of food restrictions were determined for all of the participants using the revised version of the Three-Factor Eating Questionnaire (TFEQ) (14). The 21-item TFEQ is a composite score with a scale that measures 3 domains of eating behavior: cognitive restraint, uncontrolled eating, and emotional eating. This measure has been reported to be a useful tool for characterizing these 3 domains, showing robust factor structure and good reliability (14). Subjects with TFEQ scores lower than 2.46 were selected (n = 33). These participants were randomly allocated to 1 of the 6 following sequences of control (A) and cereal-based bread consumption (B): AABB, ABAB, ABBA, BBAA, BAAB, and BABA. A simple random procedure with equal probability of being assigned to each sequence was used to allocate participants to sequence of treatment. The study was performed on 2 different days for each bread, control and cereal-based (4 test days for each subject), separated

TABLE 1 Composition of the 2 breads[1]

	Cereal-based bread[2] 1 serving	Control bread[3] 1 serving
Energy, kcal (kJ)	257 (1080)	251 (1049)
Protein, g	13.0	7.3
Total carbohydrate, g	37.8	46.9
Sugars, g	8.8	8.7
Total fat, g	3.7	3.8
Saturated fat, g	1.7	0.8
Fiber, g	10.2	2.7
Sodium, g	0.3	0.4

[1] One serving of bread (100 g for cereal-based bread; 97 g for control bread) was consumed every day of the intervention.
[2] Consisted of a well-balanced variety of cereal flours (wheat, oat, and spelt) and 22% dried fruits (figs, apricots, raisins, and prunes), and was enriched with fiber (7% added fiber consisting of wheat cross-linked maltodextrins and pea fiber, with 6.0% soluble and 4.1% insoluble fiber) and protein (10–11% added from wheat gluten and hydrolyzed wheat proteins), with no added sugar.
[3] Consisted of 85 g commercially available sliced white bread with 10 g jam and 2 g margarine to adjust for energy density, fat, and sugar levels.

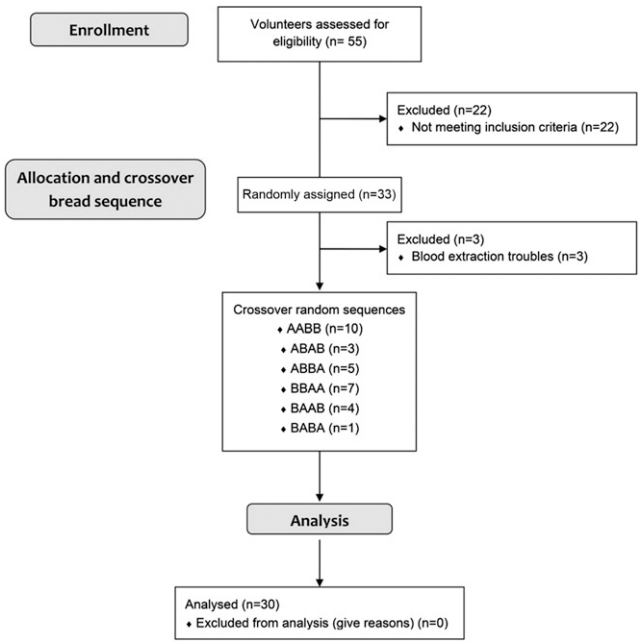

FIGURE 1 Consolidated Standards of Reporting Trials–based flow diagram of the recruitment, enrollment, and random assignment processes.

[9] Abbreviations used: GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; LMM, linear mixed-effect model; PP, pancreatic polypeptide; PYY, peptide YY; TFEQ, Three-Factor Eating Questionnaire; VAS, visual analogue scale.

by at least 1 wk (wash-out period). Group allocation was not masked. The analysts were blinded to the analyses.

Three participants were excluded because of severe difficulties with blood extraction. Thirty healthy adults (17 men and 13 women) ranging in age from 19 to 32 y (mean age 25 ± 1), with BMIs ranging from 19.2 to 28.5 (mean BMI 23.3 ± 0.5—normal to moderately overweight) participated in and completed this study. A complete clinical history of each subject was taken, including demographic data (age, sex, origin, and family history), background of disease, and current use of any drugs.

Study performance. This study was carried out according to European Food Safety Authority requirements (15). The participants were instructed to refrain from alcohol and from performing difficult physical activities 48 h before each test day. The evening before the test day, the participants consumed a standardized dinner consisting of pizza and pineapple juice (800 kcal; protein 18% of energy, fat 22% of energy, and carbohydrate 60% of energy). The participants were instructed to not eat or drink anything other than a half liter of water after the dinner.

On the morning of the test days, the participants traveled to the José Mataix Institute of Nutrition and Food Technology at the University of Granada in Spain by car, bus, or slowly walking, arriving at 8 a.m. On day 1 of the intervention and after 20 min of resting, anthropometric measurements (weight, height, and waist circumference) were measured. On each day of the study, a fasting blood sample was collected and appetite was assessed with a visual analogue scale (VAS), as described below (15).

The participants were instructed to consume either the cereal-based bread or the control white bread plus jam and margarine (to control for energy and fat intake) within 10 min, according to their randomly assigned sequence. The participants were allowed to drink only 150 mL of water with the breads. The participants immediately completed 2 VASs, one on breakfast palatability and another on appetite ratings; this VAS was repeated every 30 min until 4 h had passed.

The participants were not allowed to eat or drink anything else during the 4 h of the intervention. They were allowed to read, study, talk, or listen to quiet music, but they were not allowed to sleep. After the final blood extraction (4 h), participants consumed an ad libitum lunch consisting of a standardized Bolognese spaghetti (protein 17% of energy, fat 34% of energy, and carbohydrate 49% of energy) and water (300 mL). The participants were instructed to eat until comfortably satisfied. Food intake was registered by differences in spaghetti weight before and after lunch, and the energy intake was subsequently calculated. After the ad libitum lunch, the participants completed 2 VASs, one on meal palatability and another on appetite. The participants completed a 48-h dietary survey diary, including their food intake the day before and the day of the intervention. This study was conducted according to the guidelines set by the Declaration of Helsinki and was approved by the Ethics Committee of the University of Granada. All of the participants gave written, informed consent to participate in the study. This trial was registered at clinicaltrials.gov as NCT02090049.

Appetite profile determination. The primary outcome of the present study was the appetite profile as assessed using the VAS ratings of hunger, fullness, desire to eat, and prospective food consumption. These measures were obtained using a 100-mm scale ranging from 0 ("not at all") to 100 ("extremely") (16). This questionnaire was completed before consumption of the bread and every 30 min over the 240 min after intake of the bread. A validated composite appetite score was calculated using the following equation: Composite appetite score = [satiety + fullness + (l00 − prospective food consumption) + (100 − hunger)]/4 (16). Additionally, information regarding the appearance and palatability of the breads and lunches was recorded.

Blood sampling. Blood was collected before the intake of the products (time 0) and immediately after regular intervals the ingestion of the bread, i.e., at 15, 30, 45, 60, 90, 120, 180, and 240 min. This procedure was repeated in duplicate for each product.

Analytic methods. As secondary outcomes, we measured plasma glucose, insulin, and gastrointestinal hormone concentrations. Whole blood was added to Pefabloc SC (AEBSF) (Roche Diagnostics) and dipeptidyl-dipeptidase IV inhibitor (Millipore Iberica) for the determination of gut hormone plasma concentrations. Plasma glucose concentrations were spectrophotometrically determined using standardized commercial kits (Spinreact). Insulin, ghrelin, GIP, GLP-1, PP, and PYY concentrations were determined using a MILLIplex kit. A Luminex 200 System built on xMAP technology with the Human Gut Hormone Panel (Millipore Iberica) was used for these analyses. Cholecystokinin concentrations were determined using an enzyme immunosorbent assay kit (Ref. EKE-069–04, Phoenix Europe GmbH).

The AUCs of postprandial VAS, glucose, insulin, and gastrointestinal hormone time courses were calculated with the use of a trapezoidal method with R statistical software (17).

Statistics. The minimum sample size to detect a significant effect from the bread, based on a crossover study, was estimated to be 30 participants per group, with a type I error $\alpha = 0.05$, a type II error $\beta = 0.1$ (power 90%), and a potential drop-out rate.

Values are presented as means ± SEMs. Before any statistical analyses, all variables were checked for normality using the Shapiro-Wilk test.

TABLE 2 Baseline demographic, anthropometric, and plasma clinical characteristics of the healthy adult volunteers[1]

Characteristic	Value
Gender, M/F	17/13
Age, y	25 ± 1
BMI, kg/m^2	23.3 ± 0.5
Total water, kg	37.8 ± 1.3
Bone mass, kg	2.7 ± 0.1
Basal metabolism, kcal/d	1630 ± 50
Bioimpedance,[2] ohms	543 ± 11
Glucose, mmol/L	5.3 ± 0.1
Insulin, mIU/L	6.0 ± 0.6
TGs, mmol/L	0.8 ± 0.1
Total cholesterol, mmol/L	3.5 ± 0.1
HDL cholesterol, mmol/L	1.5 ± 0.1
LDL cholesterol, mmol/L	1.7 ± 0.1
NEFA, mmol/L	0.4 ± 0.0

[1] Values are means ± SEMs unless otherwise indicated, $n = 30$. NEFA, nonesterified fatty acid.
[2] Bioimpedance analysis was used to estimate body composition.

TABLE 3 Dietary intake and VAS scores after intake of the cereal-based bread and control bread, expressed as the AUC of postprandial curves, in healthy adult volunteers[1]

	Cereal-based bread	Control bread
Intake 24 h before,[2] kcal	2540 ± 110	2460 ± 90
Lunch eaten,[3] kcal	1000 ± 50	1020 ± 60
Intake 24 h after,[4] kcal	2180 ± 120	2260 ± 120
Hunger AUC, m · min	−5.9 ± 0.7	−4.7 ± 0.6
Satiety AUC, m · min	6.8 ± 0.8	5.9 ± 0.7
Fullness AUC, m · min	1.0 ± 0.1	6.5 ± 0.7
PC AUC, m · min	−5.3 ± 0.6*	−4.4 ± 0.6
CAS AUC, m · min	6.2 ± 0.7*	5.2 ± 0.6

[1] Values are means ± SEMs, $n = 30$. Each volunteer consumed the cereal-based bread and the control bread 2 times. The AUC was calculated for postprandial time course 4-h curves. A negative value indicates a postprandial decrease (negative response). The LMM was applied to individual measurements from participants to calculate *P*-values. *Different from control, $P < 0.05$. CAS, composite appetite score; LMM, linear mixed-effects model; PC, prospective consumption; VAS, visual analogue scale.
[2] Dietary intake 24 h before the day of the intervention.
[3] Ad libitum intake 4 h after the intervention.
[4] Dietary intake 24 h after the intervention.

TABLE 4 Correlations between appetite scores 240 min after consumption of the cereal-based or control breads and ad libitum energy intake[1]

	Global	Cereal-based bread	Control bread
Hunger	0.45‡	0.30‡	0.14†
Satiety	−0.44‡	0.30‡	0.13†
Fullness	−0.39‡	0.19†	0.13†
PC	0.53‡	0.30‡	0.30†
CAS	−0.48‡	0.29‡	0.18‡

[1] Values of Pearson's *r* coefficient are indicated. The correlations were calculated using all of the data (global) and by bread type. There were relations between all appetite-rating scores immediately before the ad libitum lunch and hormone levels at the same time point. Each volunteer consumed the cereal-based bread and the control bread 2 times. The AUC was calculated for the postprandial time course 4-h curves. Symbols indicate significant correlations: †$P < 0.01$; ‡$P < 0.001$. CAS, composite appetite score; PC, prospective consumption.

Homogeneity of the variances was estimated using Levene's test. Glucose did not meet the normality assumption; thus, log-transformed data were analyzed. To determine differences between treatments at each postprandial time point, a *t* test for dependent data (paired sample *t* test) was carried out to check the hypothesis of equal means between the 2 breads. The carryover effect was checked and found not to be significant ($P \geq 0.05$; results not shown). A linear mixed-effects model (LMM) was used to compare AUCs calculated for both breads. This method analyzes repeated measures over time and considers the relation of responses within participants. The chosen fixed effects were treatment, age, gender, BMI, and the day of intervention (period effect); these effects were analyzed and eliminated if there were no significant changes (results not shown). The advantages of the LMM vs. the ANOVA for repeated measures in these types of studies have been reported elsewhere (18). The LMM provides a more precise estimation of covariances and does not require mathematical assumptions, unlike the ANOVA. The means of the 2 measures for each treatment were used to calculate descriptive statistics and perform paired *t* tests. However, *P*-values in the tables are from the adjusted LMMs, which were applied to individual measurements (n = 2/treatment) from the 30 participants. Within the LMMs, the factor treatment, time, and random effect for each participant takes into account the structure of the data. Thus, results adjusted by those models are more robust and reliable. The Pearson's correlation coefficient was used to test the associations between energy intake, appetite scores, and gastrointestinal hormones. $P < 0.05$ was considered significant. SPSS version 20 was used to perform the statistical analysis.

Results

Baseline subject characteristics. All of the participants completed the 4 test days according to the protocol. The BMI, body fat, total water, bone mass, basal metabolism, and body bioimpedance analysis, used to estimate body composition, were within normal ranges (19, 20). Similarly, plasma glucose, insulin, TGs, total cholesterol, LDL and HDL cholesterol, and nonesterified fatty acid concentrations were normal (**Table 2**). No significant differences in weight or fasting concentrations for the measured variables were observed during the time of the study.

Appetite ratings. Ad libitum energy intake 4 h after ingestion of the breads, as well as AUCs of all of the variables used to determine the appetite ratings, are shown in **Table 3**. There were no differences in the amount of spaghetti eaten 4 h after the ingestion of the breads; however, the LMM indicated that the postprandial hunger decrease (negative AUC) tended to be greater after the intake of the cereal-based bread (P = 0.06), whereas the prospective consumption decrease (negative AUC) was higher (P = 0.02). In addition, the composite appetite score, a global measure of satiety, was higher after intake of the cereal-based bread (P = 0.04). No significant differences were observed in the AUC values of satiety and fullness after ingestion of the 2 types of breads (Table 3). At 240 min after consumption of the 2 breads, there were significant direct relations between the amount of lunch eaten, hunger, and prospective consumption; also at this time point, satiety and fullness both were inversely related to the composite appetite score (**Table 4**).

Glucose, insulin, and gastrointestinal hormone responses. The time course for plasma glucose, insulin, and gastrointestinal hormone concentrations after the ingestion of either the cereal-based or the control bread is depicted in **Figures 2** and **3**. The AUCs for these variables are shown in **Table 5**. Volunteers who ingested the cereal-based bread had a lower postprandial glycemic and insulinemic response than those who ingested the isocaloric control bread (Figures 2A and 2B, respectively, and Table 5). The postprandial decrease in the secretion of ghrelin (AUC) was greater after ingestion of the control bread than after ingestion of the cereal-based bread, as determined by lower plasma concentrations from 30 to 120 min (Figure 3A and Table 5). GLP-1 plasma concentrations were lower 45 min after the intake of the cereal-based bread compared with control, but higher 240 min after consumption (Figure 3B). In addition, the GLP-1 AUC was significantly greater after ingestion of the control bread (Table 5). After ingestion of the cereal-based bread, the postprandial release of GIP was lower from 30 to 90 min compared with the control bread (Figure 3C), with a significantly lower AUC (Table 5). The PP AUC and PP plasma concentrations at 60, 90, and 120 min were significantly higher after consumption of the cereal-based bread (Figure 3D) (Table 5). Finally, PYY and cholecystokinin secretion did not differ after the ingestion of either bread (Table 5).

A smaller ad libitum lunch size was positively associated with a smaller variation in plasma ghrelin and a greater increase in GIP, PP, and cholecystokinin AUCs (**Table 6**). The postprandial

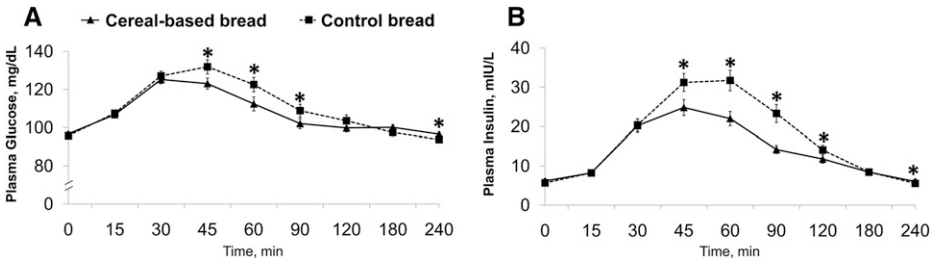

FIGURE 2 Postprandial time courses of glucose (A) and insulin (B) plasma concentrations after the intake of the cereal-based bread and the control bread in healthy adult volunteers. Each volunteer consumed the 2 different breads 2 times, and the mean of the 2 measures was used. Values are means ± SEMs, n = 30. A paired *t* test was carried out to determine differences between treatments at each postprandial time point. *Different from control, $P < 0.05$.

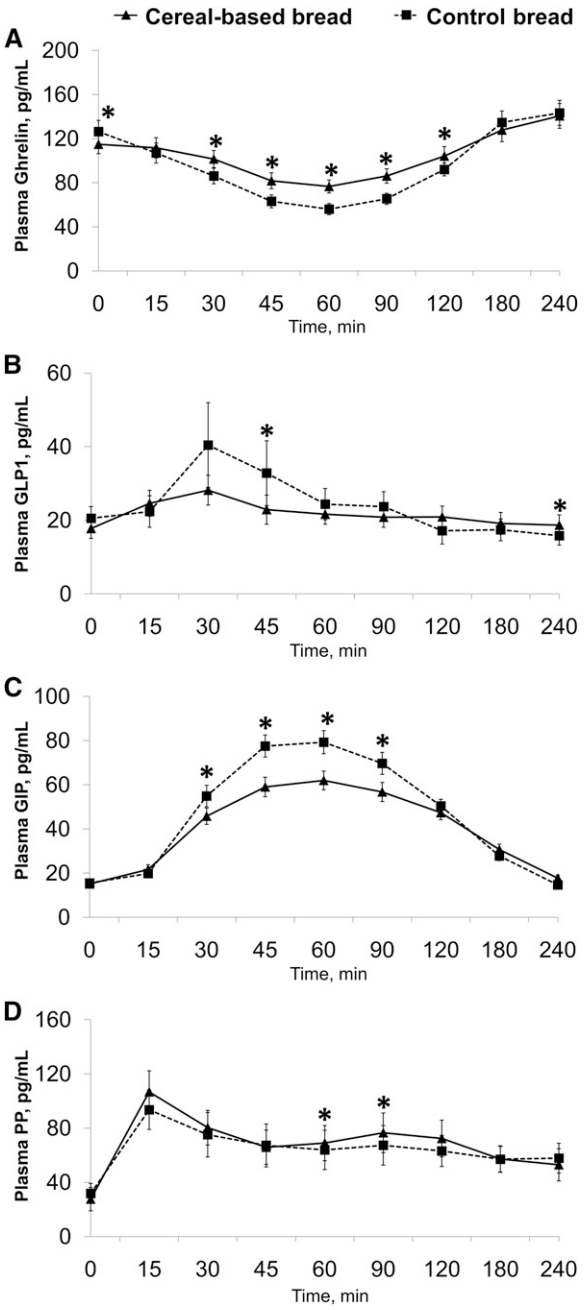

FIGURE 3 Postprandial time courses of ghrelin (A), GIP (B), GLP-1 (C), and PP (D) plasma concentrations after the intake of the cereal-based bread and the control bread in healthy adult volunteers. Each volunteer consumed the 2 breads 2 times, and the mean of the 2 measures was used. Values are means ± SEMs, $n = 30$. A paired t test was carried out to determine differences between treatments at each postprandial time point. *Different from control, $P < 0.05$. GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; PP, pancreatic polypeptide.

drop in GLP-1 and PYY were not associated with energy intake. In addition, there were relations between all appetite rating scores immediately before the ad libitum lunch and GIP, PP, PYY, and cholecystokinin AUCs (Table 6). Although the ghrelin AUC was not associated with appetite ratings immediately before the ad libitum lunch, we found that the ghrelin AUC correlated significantly with a global variation in appetite scores: directly with hunger AUC ($r = 0.25$, $P = 0.006$) and inversely with satiety AUC ($r = -0.22$, $P = 0.002$) and composite appetite score ($r = -0.19$, $P = 0.04$).

TABLE 5 Plasma glucose, insulin and gastrointestinal hormone concentrations in healthy adults after intake of the cereal-based and control breads expressed as the AUC of postprandial curves[1]

	Cereal-based bread	Control bread
Glucose AUC, mg/dL · min	2.3 ± 0.3**	3.1 ± 0.4
Insulin AUC, mIU/L · min	1.5 ± 0.1***	2.3 ± 0.2
Ghrelin AUC, pg/mL · min	−4.1 ± 0.6***	−7.9 ± 0.9
GIP AUC, pg/mL · min	6.0 ± 0.5**	6.9 ± 0.5
GLP-1 AUC, pg/mL · min	1.0 ± 0.3*	1.1 ± 0.4
PP AUC, pg/mL · min	9.4 ± 1.6**	7.7 ± 1.8
PYY AUC, pg/mL · min	1.0 ± 0.2	1.0 ± 0.2
CCK AUC, μg/L · min	4.9 ± 0.8	4.3 ± 0.9

[1] Values are means ± SEs, $n = 30$. Each volunteer consumed the cereal-based bread and the control bread 2 times. The AUC was calculated for the postprandial time course 4-h curves. A negative value indicates a postprandial decrease (negative response). The LMM was applied to individual measurements from participants to calculate P values. Asterisks indicate different from control: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. CCK, cholecystokinin; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; LMM, linear mixed-effects model; PP, pancreatic polypeptide; PYY, peptide YY.

Discussion

The most relevant findings of the present study were that intake of the cereal-based bread decreased postprandial prospective consumption and increased satiety, measured as a composite appetite score, compared with the control bread. The cereal-based bread exerted a clear effect on physiologic mechanisms related to satiety as determined by changes in gastrointestinal hormone plasma concentrations. We demonstrated a clear relation between modifications of appetite scores and gastrointestinal hormones with the decrease in food intake. Furthermore, postprandial blood glucose and insulin concentrations were lower after ingestion of the cereal-based bread. These effects primarily can be explained not only by the higher fiber content in the cereal-based bread but also by the presence of added proteins. Many studies have described the satiating effect of dietary fiber (12, 21, 22) and different sources of proteins (23–26).

We observed a higher fullness and satiety score for the cereal-based bread; however, this effect was not accompanied by a significant reduction in food intake at the ad libitum lunch. This often occurs because food cannot be expected to act like a drug (27). Indeed, other authors have described that increased ratings of satiety and fullness were not accompanied by a decrease in subsequent energy intake (13, 28). These findings were likely because of other physiologic effects (13, 29), as well as psychological and environmental factors that influence food intake (15). In addition, different types of dietary fiber and other nutrients such as proteins present in the meal may have different influences on physiologic activities (13, 30). Although no reduction in ad libitum food intake was found, we observed a relation between all appetite scores and ad libitum energy intake that support the satiating effect of the cereal-based bread. In the context of a food matrix, functional ingredient activities may be modulated by other components.

Several mechanisms have been suggested for how dietary fiber aids in weight management, such as promoting satiety, decreasing the absorption of macronutrients, and altering the secretion of gut hormones (29, 30). Indeed, dietary fiber reduces the energy density of foods because it is not absorbed in the small intestine. Fiber also slows the absorption of carbohydrates by increasing digestion viscosity, delaying gastric emptying, and/or shortening transit time through the small intestine (12, 31). In

TABLE 6 Correlations between appetite scores at 240 min after intake of the cereal-based and control breads, as well as ad libitum energy intake, with gastrointestinal hormone AUCs obtained from all of the data[1]

	Ghrelin	GLP-1	GIP	PP	PYY	CCK
Energy intake	−0.21[§]	0.27	−0.54[‡]	−0.34[†]	−0.02	−0.36[†]
Hunger	−0.12	−0.16	−0.27[†]	−0.37[‡]	−0.24[†]	−0.19[§]
Satiety	0.015	0.013	0.28[†]	0.38[‡]	0.28[§]	0.27[§]
Fullness	0.08	0.07	0.16[†]	0.28[†]	0.26[§]	0.20[§]
PC	−0.10	−0.12	−0.38[‡]	−0.4[‡]	−0.27[†]	−0.24[§]
CAS	0.12	0.10	0.29[†]	0.38[‡]	0.28[§]	0.24

[1] Values of Pearson's *r* coefficient for global correlations calculated from all of the data (not by group). There were relations between all of the appetite rating scores immediately before the ad libitum lunch and hormone AUCs. Each volunteer consumed the cereal-based bread and the control bread 2 times. The AUC was calculated for the postprandial time course 4-h curves. Symbols indicate that correlations are significant: [§]$P < 0.05$; [†]$P < 0.01$; [‡]$P < 0.001$. CAS, composite appetite score; CCK, cholecystokinin; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; PC, prospective consumption; PP, pancreatic polypeptide; PYY, peptide YY.

addition, protein has the highest satiating effect, which may be related to the altered production of appetite-regulating gastrointestinal peptides, such as the orexigenic ghrelin and anorexigenic GLP-1, PYY, and cholecystokinin (31–33). All of these effects seem to be dependent on the amount and chemical composition of dietary fiber and proteins (34, 35). In fact, dietary fiber exhibits a variety of in vivo responses that are most likely related to the significant variability in their chemical and physical attributes (12, 36, 37).

The effects of dietary fiber and proteins on ghrelin (34, 35, 38), GLP-1 (32, 34, 35, 39, 40), GIP (40), PP (41–43), and PYY (33, 35, 36, 44) secretions are unclear and depend on the amount and type of fiber and proteins. However, some authors have observed that amount of dietary protein does not affect ghrelin secretion (32). Our cereal-based bread provided 10 g of fiber and 13 g of protein, which may be responsible for the lower postprandial decline in plasma ghrelin response. Fiber-enriched breads have been reported to reduce GIP secretion compared with control white bread (35). In the present study, the main fiber component was cross-linked maltodextrins (type IV resistant starch), which is a special type of soluble fiber. In agreement with our results, Raben et al. (40) found that resistant starch resulted in lower GIP and GLP-1 responses, and a relation was described between hunger and GIP.

PP delays gastric emptying and reduces appetite ratings (43, 45), influencing the feeling of decreased hunger observed after bread consumption. This fact is confirmed by the relations found between PP and ad libitum lunch intake and appetite scores. PYY concentrations rise postprandially in proportion to the amount of dietary proteins (32, 44) and were reported to be decreased by dietary fiber (36). In agreement with Karhunen et al. (35), we did not observe differences in the postprandial PYY response; however, the relation between PYY concentrations and ad libitum lunch intake found in our study agree with the described anorexigenic effect of this peptide (33). Moreover, cholecystokinin promotes fat and protein digestion and plays a role in appetite regulation (5), results that are in agreement with our findings showing that cholecystokinin postprandial release was correlated to the ad libitum lunch eaten and all appetite scores. Although there is convincing support for the role of cholecystokinin in mediating postprandial satiety (31), we did not observe differences between the breads with respect to this metric. Because fat is the main inductor of cholecystokinin release, it is likely that this null effect is due to the equally low fat content in both of the breads (~14% of energy).

The present study indicates that ingestion of the cereal-based bread was able to diminish the postprandial increase of blood glucose and insulin compared with ingestion of the control bread. In addition, dietary proteins have an insulinotropic effect (46) lower than glucose and other bioavailable carbohydrates, and intake of soluble dietary fiber has been negatively correlated with postprandial glucose and insulin responses (34, 47–49). These effects may be related to the increased viscosity of the meal bolus in the stomach, reducing the mixing of the food with digestive enzymes and gastric emptying. These effects would delay the digestion of starch (50) and, therefore, the absorption of glucose (48). However, the decrease in incretin, GIP, and GLP-1 concentrations found in the present study may also explain the decrease in insulin release. Therefore, a direct effect of fiber and protein on gastric emptying and an indirect effect due to gastrointestinal peptides possibly influence glycemia and insulin secretion.

There is an apparent paradox in the present study in that consumption of the cereal-based bread led to increased satiety but to a lower release of GLP-1, which would be expected to show higher concentrations. It is difficult to explain the satiety effects exclusively through individual hormone effects because satiety is a complex process with many components that interact in a complementary manner (15). In addition, Gibbons et al. (27), proposed that there is neither a unique satiety hormone nor any unique profile of hormones. Consequently, identifying a specific role for each hormone may be an untenable goal.

Our breads, however, accounted for only 10% of total daily energy requirements. We preferred to administer only the cereal-based bread so that the satiating effect could not be modified or mitigated by other breakfast foods. Taking all of the results together, we have demonstrated that the cereal-based bread modified appetite ratings and gastrointestinal hormones, regulating energy intake. However, there are other factors involved in the control of food intake, such as psychological and environmental effects, that we cannot measure. In addition, energy regulation is based on learning mechanisms and repetition. For these reasons, it would be interesting to conduct a sustained intervention to evaluate the chronic effect of the cereal-based bread.

In conclusion, the consumption of a cereal-based bread enriched in fiber and proteins contributes to appetite control by reducing hunger, enhancing feelings of satiety, and improving glycemia, insulinemia, and gastrointestinal hormone responses. These widely considered healthy effects may be beneficial for the prevention and treatment of metabolic diseases. Further exploration of these results will be useful in improving our knowledge of how different fiber- and protein-enriched foods, specifically breads, may influence appetite ratings and release of gastrointestinal hormones.

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