Official Title of the study: Effects of Barley and Oat Breads on Appetite

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1. Background

The prevalence of overweight and obesity worldwide increases significantly with concomitant diseases. Disruption of energy balance is known as the main cause of obesity. To maintain energy balance, weight control (dietary intervention, physical activity, behavioral therapy) results in short-term weight loss, but sustained weight maintenance is difficult. So alternative weight-loss strategies can be a solution (1). Epidemiological studies show that high dietary fiber intake is associated with lower body weight and waist circumference (2,3). The controlled intervention studies report that weight gain can be prevented by reducing appetite and energy intake with high dietary fiber intake (4–7). In addition, most studies show that high fiber intake is also associated with improved postprandial glycemic response (8). These potential beneficial effects of dietary fiber, which is divided into two as soluble and insoluble according to its physiological effects, can be attributed to ß-glucan, a type of soluble fiber (9). However, results from studies examining the effects of ß-glucan on appetite are not consistent and the underlying mechanisms are not yet clear. It is thought to contribute to a feeling of satiety by increasing the duration of oral exposure to food and abdominal distension and by increasing short-chain fatty acid production as a result of colonic fermentation (10–12). The hypothesis that ß-glucan affects appetite by acting on gastrointestinal hormones is still controversial (13). On the other hand, the blood-glucose-lowering properties of ß-glucan derived from oats and barley have been confirmed by the European Food Safety Authority (EFSA), with a health claim that 4 g ß-glucan can be used per 30 g of available carbohydrates per meal (14). The lowering effects of ß-glucan on blood glucose and insulin levels and blood pressure are speculated to be due to its prebiotic properties and its effects on weight loss (9).

Overall, although studies have shown positive effects of ß-glucan on appetite and postprandial glycemic response, it is not yet clear whether this positive effect is due to ß-glucan or total dietary fiber intake. Also, since the majority of studies used products enriched with ß-glucan, little is known about the possible effects of dietary sources with ß-glucan in their natural form. In this study, the effects of breads derived from barley and oat, which are the best grain sources of ß-glucan, on appetite and postprandial glycemia were examined and the possible effects of its natural dietary sources were determined. In addition, the effects of breads, which are a source of ß-glucan, on appetite and postprandial glycemia were evaluated by comparing not only with white bread (WB) but also with whole wheat bread (WWB), unlike previous studies. In this way, it was aimed to determine whether the possible positive effects were due to ß-glucan or total dietary fiber intake. The selection of bread, which is the most commonly consumed grain-based food in European countries, as the test food will make it easier to add possible positive results to healthy eating recommendations and to their applicability.

2. Objective

This study aimed to compare the postprandial effects of ß-glucan on glycemia and appetite of breads derived from oats and barley, which are the best natural grain sources. In addition, the positive health effects of ß-glucan were sought to be determined independent of total dietary fiber intake, using not only WB as the control food but also WWB, which has a dietary fiber content equal to that of breads containing ß-glucan.

3. Materials and methods

3.1. Participants and Study Design

This study was planned as a multicenter, randomized, double-blind, and crossover. Approval was obtained from the Erciyes University Clinical Research Ethics Committee for the study (Decision No: 2018/259, Date: 09.05.2018). Twenty healthy adults (10 men, 10 women) between the ages of 19-35, with normal body weight (BMI 18.5-25 kg /m^2) were included in the study. Exclusion criteria were applying an energy-restricted diet in the last three months, having a change in body weight >5 kg in the last three months, having a chronic disease such as diabetes and hypertension, or using a drug that affects the metabolism, having fasting glucose >100 mg/dl, smoking, exercising heavily, having chewing/swallowing difficulties or sensitivity/allergies to any food to be used in the study, vegans, pregnant and lactating women, and skipping breakfast meals. Participants were selected from among Erciyes University students and staff in accordance with the inclusion criteria. Before the participants were included in the study, they were informed about the study in accordance with the Declaration of Helsinki, and volunteers were asked to read and sign the informed consent form.

The physical activity levels of the participants were evaluated simultaneously with dietary assessment by the International Physical Activity Questionnaire (IPAQ) short form at the beginning of the study and on each test day. Their dietary intakes were also assessed by the a-24-hour dietary recall using a photographic atlas of food portion sizes to quantify the data. Diet composition was analysed by the BeBiS Nutrition Information System software version 8.2. Furthermore, body weight and height of participants were obtained at the beginning of the study, and BMI was calculated. Waist and hip circumference were measured using a non-elastic tape with the participants standing, with the face directed towards, shoulders relaxed, and the tape was positioned at a level parallel to the floor. The eating behaviors of all participants were also evaluated using the Dutch Eating Behavior Questionnaire before starting the study. Since the total score obtained from this questionnaire >3.5 is considered as restrictive eating behavior (15), these individuals were not included in the study.

Since individuals were asked to fast for at least 12 hours before consuming the test meals and the postprandial response was more pronounced in the morning, breakfast was chosen as the test meal. On the day before each test meal, the participants were asked to consume their standard dinner at 20:00 and not consume any food or beverage (except water) after dinner, and not to do any physical activity other than their daily routine activities. In addition, test meals were consumed in the follicular phase of the menstrual cycle in order to control the possible effects of the menstrual cycle on appetite for female participants. Similar to previous studies (16,17), days 3-10 after the onset of menses were accepted as the follicular phase.

Participants were served barley bread (BB), oat bread (OB), and wheat breads (WB and WWB) with a standard breakfast on four different days at least two days apart (18). Breads were prepared with similar amounts of energy, protein, fat, carbohydrate, and fiber (except WB). The test meal was served to the participants at 09:00 and they were asked to consume it within 15 minutes. During the test period, participants were not allowed to eat or drink anything other

than water. On the first day of testing, participants were able to consume as much water as they wanted and the amount consumed was recorded. On the other test days, they were asked to consume the amount of water recorded on the first test day. In addition, the participants were allowed to watch movies, read books, play games on electronic devices (computer, cell phone, etc.), and do other sedentary activities during the test days, but they were not allowed to sleep.

3.2.Chemical Analysis

In this research, total fat, protein, ß-glucan, total available carbohydrate, and total dietary fiber analyzes were performed chemically in the flour and bread samples. Chemical analyzes were performed in parallel and in duplicate. Data were reported on dry matter and average values of at least two analyzes were taken. The energy value (kcal) per test portion was calculated using the weight (g) of each test portion and the formula [(4 x protein) + (9 x fat) + (4 x available carbohydrate)] (19). All chemical analyzes were conducted in Erciyes University Veterinary Faculty Food Hygiene and Technology Laboratory.

All chemical analysis was performed as described above by taking a sample of several flours commonly available in Turkey. Among these flours, barley and oat flours with the highest ß-glucan content were selected for baking bread as well as white flour and whole wheat flour having the most similar macronutrient content with barley and oat flours. A preliminary study was conducted to evaluate β-glucan content and bread baking tests were applied. For BB and OB, the content of β-glucan was targeted to be about 3 g in one serving. WB and WWB were used as reference and control foods, respectively. After the test breads were prepared, all the chemical analyzes described above were carried out again and the nutritional composition of the breads was found as in Table 1.

Table 1. Energy and macronutrient contents of test breads

	White bread	Whole-wheat bread	Barley bread	Oat bread
Portion size (g)	72	73	73	70
Energy (kcal)	150	147	154	133
Carbohydrate (g)	28.7	27.3	28.9	23.9
Protein (g)	5.0	5.7	4.9	4.7
Fat (g)	1.7	1.7	2.1	2.0
Dietary fiber (g)	2.2	6.8	6.9	9.0
β-glucan (g)	1.2	1.4	3.1	3.2

3.3.Baking The Test Breads

For WB, WWB, BB, and OB, baking tests were performed using 100% white flour, whole wheat flour, barley flour, and oat flour, respectively. All breads have similar ingredients except the types of flour used. Dry yeast (2 g / 100 g), sugar (4 g / 100 g), salt (2 g / 100 g), and sunflower oil (4 g) in all breads were added (based on 100 g flour) (20). After resting for 30 minutes at room temperature (25 °C), the samples were fired in an electric oven at 180 °C for

30 minutes (21)(20). One serving size slices were wrapped in aluminum foil and packed in plastic bags to prevent drying and stored at -18 °C until use (22).

3.4.Test meals

The foods and nutritional composition of the test breakfast meals are as in Table 2.

Table 2. Nutritional composition of the component foods in test meals

Foods	Portion size (g)	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)
Feta cheese	15	34.8	0.66	1.8	2.7
Cheddar cheese	24	79.2	0.48	5.28	6.24
Olive	24	57.1	0.30	0.32	4.79
Honey	20	64	16.6	0.06	-
Black tea	150 ml	-	-	-	-

3.5.Postprandial Appetite and Glucose Assessment

A visual analog scale (VAS) was used to assess appetite before breakfast and at 15, 30, 60, 90, 120, 150, and 180 minutes after breakfast (23). Furthermore, the separate VAS components such as hunger, fullness, desire to eat, and prospective food consumption were combined to produce an additional measure termed ‘composite appetite score’. This validated average appetite score was calculated for each time point using the following equation: [(hunger + desire to eat + prospective food consumption + (100 - fullness)]/4 (24).

For the postprandial glucose assessment, blood glucose measurements were made using a finger-tip glucometer (Accu-Check, Roche Diagnostics) at 0, 15, 30, 45, 60, 90, and 120 minutes.

3.6.Ad libitum Lunch

At the end of three hours, the ad libitum lunch was served to the participants as a buffet-style, and the energy and macronutrient intakes in this meal were determined. All foods (meatballs, tuna, sauce pasta, yogurt, salad, white bread, apple, banana, yogurt drink, fruit juice, and water) were weighed using a calibrated kitchen scale with an accuracy of ±1g before being served. At lunch, the participants were asked to consume the foods they wanted and continue eating until they felt completely full. After the participants completed their consumption, the remaining amounts were reweighed and the difference with the initial amounts was accepted as the amount consumed. Energy and macronutrient values were calculated using The National Food Composition Database (TurKomp), and manufacturer labelling.

4. Statistical Analysis Plan

4.1. *Sample size*

A power-based sample size calculation based on previous research from Bo et al. (25) revealed that 16 participants were needed to provide 80% power to detect 5% difference between groups in primary outcomes. To allow discontinuation during the study, 20 participants (considering 25% losses) were recruited.

4.2. Data analysis

Statistical analysis was performed using the SPSS Statistics (version 22.0) software. Data were expressed as the mean ± SD unless otherwise indicated. Normality was assessed using the histogram and normal Q-Q plots, and also Shapiro-Wilk test. Postprandial appetite and glucose responses were quantified as incremental area under the curve (iAUC) calculated according to the trapezoidal rule. One-way analysis of variance (ANOVA) for repeated measures was applied to determine statistical differences between groups. For all statistical analyses, p values less than 0.05 were considered to have statistical significance.

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