BIOAg Project Report

Report Type:
Final

Title:
Human Health from Soil to Society: Barley β-glucan, glycemic control, and appetite

Principal Investigator(s) and Cooperator(s):
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Abstract:
The effects of high β-glucan biofortified barley (*Hordeum vulgare* L.) on post-prandial blood glucose response, appetite, and energy intake were evaluated in a randomized, controlled, crossover trial. The research aims were to (1) determine the response of different dosages of β-glucan on post-prandial blood glucose, appetite, and energy intake; and (2) assess the ability of barley β-glucan to attenuate glycemic response to ingestion of high-sugar foods. Sixteen healthy male and female participants ages 22-38 years attended four or eight separate laboratory testing sessions spaced approximately one week apart. At 8:00 am, and every 15 minutes thereafter, blood glucose was measured via finger prick with a point-of-care glucometer and participants provided subjective appetite ratings with a 100-mm Visual Analog Scale (VAS) displayed on a handheld tablet. At 8:00 am, participants consumed a porridge preload (240 mL) of an unsweetened condition (250 kcal): glutinous white rice (WR), low β-glucan barley (LB), medium β-glucan barley (MB), or high β-glucan barley (HB); or a sweetened condition with high fructose corn syrup (HFCS) (391 kcal): WR + HFCS, LB + HFCS, MB + HFCS, or HB + HFCS. A 16-item test lunch was provided at 12:00 pm, from which participants consumed *ad libitum.* Calculations included (1) incremental area under the curve for blood glucose over multiple periods (1-4 hours); (2) area under the curve for self-reported appetite ratings over multiple periods; and (3) energy intake and the weight of foods and water consumed for test lunch food intake. Statistically significant findings were found for all three outcomes (blood glucose response, subjective appetite ratings and test lunch intake) under the unsweetened porridge preload conditions.

Project Description:
We evaluated the effects of β-glucan barley on short -term glycemic control and appetite. To achieve our specific aims, we conducted a randomized cross-over human clinical trial. Each participant served as their own control, reducing both variation and required sample size.

On each study day, participants visited Dr. Perrigue's Food Lab for a 4.75-hour testing session. Participants arrived at 7:45 am after an overnight (10-12 hr.) fast. At baseline (8:00 am) and every 15 minutes thereafter, participants completed appetite ratings using computerized Visual Analogue Scales (VAS) and tested their own blood glucose by using a finger prick and glucometer. A total of 17 finger pricks were collected every 15 minutes between 8:00 am and 12:00 pm. At 8:00 am, directly after the first appetite rating and blood glucose measurement, participants consumed a barley porridge preload that may or may not have included corn syrup.

Participants that completed the first four weeks of the trial consumed one of four equal-volume products varied in β-glucan content: a) glutinous white rice (WR); b) low β-glucan barley (LB); c) medium β-glucan barley (MB); or d) high β-glucan barley (HB). Participants that continued for an additional four weeks, or were recruited after former participants dropped out, consumed one of four equal-volume products varied in β-glucan content and corn syrup: a) WR + HFCS; b) LB + HFCS; c) MB + HFCS; d) or HB + HFCS.

Each preload provided approximately 240 mL of food (250 kcal unsweetened; 391 kcal. sweetened). Participants were provided with 12 fluid ounces of water with the preload. After the blood glucose measurement and appetite rating at 12:00 pm, participants were given a test lunch, from which they were instructed to eat *ad libitum*. The test lunch included fruits, vegetables, hummus, deli meats, cheese, yogurt, and desserts. The participants were allowed 30 minutes to eat. At 12:30 PM, participants completed a final appetite rating (no blood glucose measurement). Participants were free to leave the laboratory immediately following the final appetite rating at 12:30 pm. The meal components were weighed when the participant left.

Outputs

Overview of Work Completed

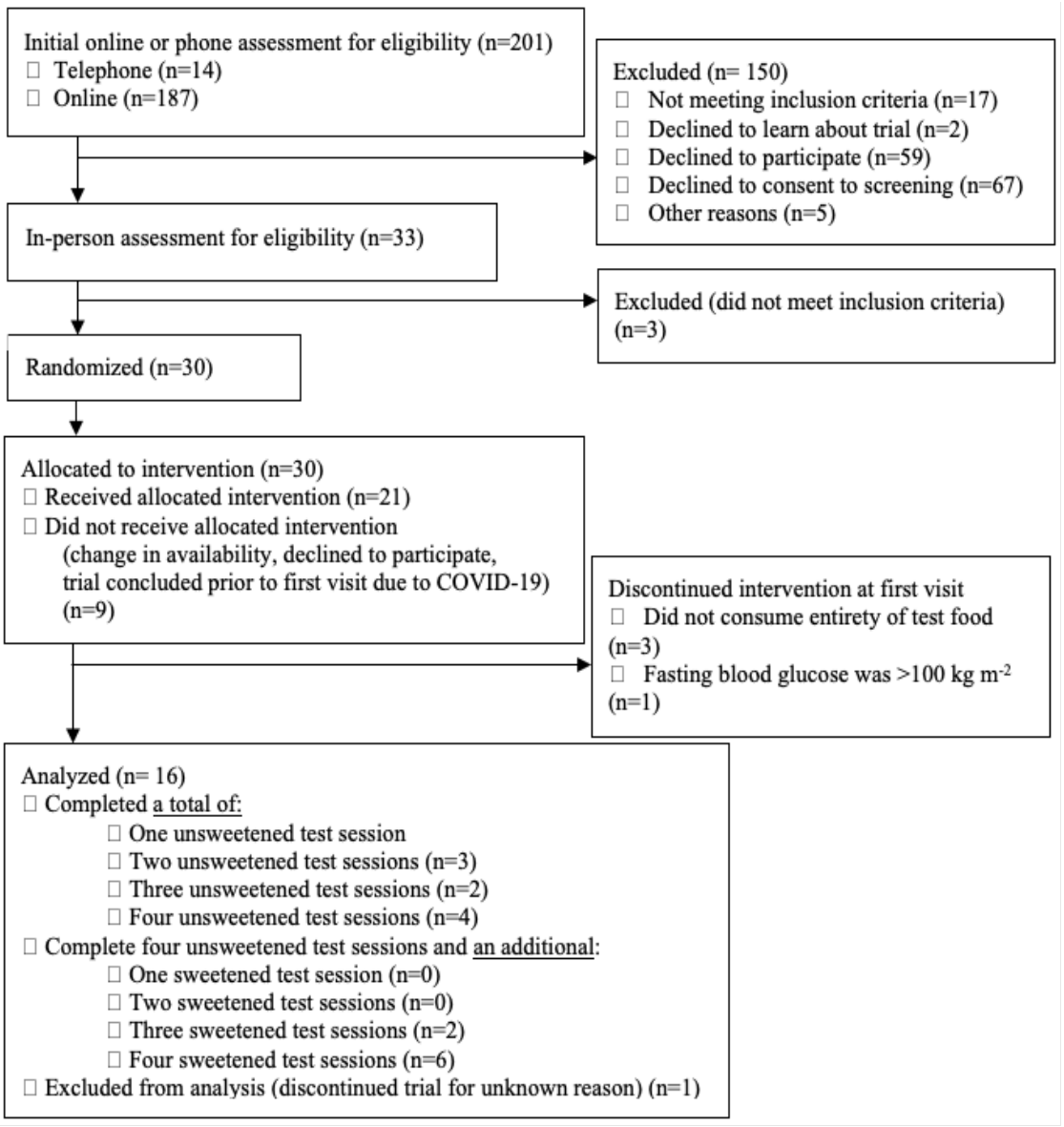
Over the first 6 months of grant funding, all necessary equipment was acquired to set up the clinical lab located in the Health Sciences Building in room 320R. This room was completely empty prior to commencing this study. The lab is now capable of running four human subjects at a time (Fig. 1).

Figure 1. WSU Elson S Floyd College of Medicine Perrigue Food Lab layout. Photo by Julianne Kellogg.

Additionally, the IRB application for this study was approved, a website was created (labs.wsu.edu/foodlab). Each grain used in this trial was analyzed for gross energy, total dietary fiber, protein, amylose, and β-glucan.

Participants were screened and received the intervention between the months of November 2019 and March 2020. See consort diagram for screenings and other study procedures completed (Fig. 2). Trial was concluded in March 2020 following the WSU requirement to suspend human subject research. Data was analyzed and a manuscript is in draft for submission to a peer-reviewed journal.

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) flow diagram to display participant allocation to trial treatments and participant retention. Figure by Julianne Kellogg.



To strengthen predictions of β-glucan and protein using Dr. Murphy's NIR equipment, 224 barley samples were analyzed using the NIR with 132 also analyzed using enzymatic assays for β-glucan determination. 132 of these samples were run through the Leco protein analyzer located at the Western Wheat Quality lab.

Methods

Grain analysis

Grains included in the study were analyzed for (1) gross energy using a bomb calorimeter; (2) crude protein using a Leco protein analyzer (dumas method); (3) total dietary fiber using a Sigma enzymatic and gravimetric assay; (4) β-glucan using a Megazyme enzymatic assay; and (5) amylose using a Megazyme enzymatic assay.

Participant visit schedule

On each study day, participants visited Dr. Perrigue's Food Lab for a 4.75-hour testing session (Fig.3)

Figure 3. Schedule followed by participants at each visit to the WSU Food Lab. Figure by Julianne Kellogg.

0745 hr
- Participants arrive after overnight fast (≥10 hr)
- Participants complete check-in survey

0800 hr
- Participants record baseline (fasting) self-reported appetite ratings with Visual Analogue Scales (VAS) on electronic tablets and test blood glucose using finger prick and glucometer
- Preloads, 355 mL water served on a tray
- Participants allowed 15 min to consume all food and drink

0815 hr
- Researchers collect trays and verify complete consumption of preload and water

0830 – 1200 hr
- Every 15 min, participants report appetite ratings and blood glucose for a total of 17 data points between 0800-1200 hr for each participant

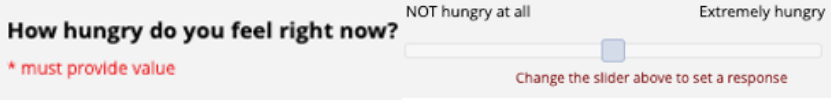
1200 hr
- Participants given 16-item test lunch, instructed to eat *ad libitum*

1230 hr
- Participants complete final self-reported appetite rating
- Excess food, water, trash collected and weighed

Measurements

The three outcomes measured in this study were post-prandial blood glucose, subjective appetite ratings, and test lunch intake. To measure blood glucose, disposable safety lancets and point-of-care glucometers were used. To measure subjective appetite ratings, Visual Analog Scales (Fig. 4) were used to assess each appetite category (hunger, fullness, desire to eat, nausea, and thirst). To measure test lunch intake, all foods and water in the test lunch were pre-weighed and served on individual trays. Each participant's tray consisted of crackers, pita bread, banana, apple slices, carrots, sugar snap peas, cheddar cheese, provolone cheese, yogurt, ham and turkey deli slices, hummus, chocolate candies, a grain and marshmallow snack, potato chips, and sherbet. This meal was designed to provide a wide variety of commonly consumed lunch items. Although participants were instructed to eat *ad libitum*, the entire meal provided approximately 1560 kcal, 197 g carbohydrates, 52 g protein, 60 g fat, 83 g sugar, and 16 g fiber. At the end of the lunch period, researchers collected the lunch trays and weighed all remaining food items to determine the amount consumed

Figure 4. Visual Analog Scale (VAS) used by participants for self-reported appetite ratings.



Data analysis
Data was managed using: REDCap (Research Electronic Data Capture) tools hosted by Washington State University. Calculations included: (1) for blood glucose, incremental area under the time curve (iAUC) calculated over multiple periods (1-4 hours) according to the trapezoidal rule with the area below the baseline fasting levels ignored; (2) for subjective appetite ratings, total area under the curve (AUC) calculated over multiple periods (1-4 hours); and (3) for test lunch food intake, total energy intake and the weight of foods and water consumed.

Results
The trial participants included in the final analysis were 16 healthy adults (Table 1). The calculated required sample size for this study was determined to be 23 participants and the study aimed for a minimum of 32 enrolled participants. However, due to the early conclusion of the trial, only 16 participants were included in the study and the final data set was unbalanced.

Table 1. Participant characteristics by gender (n = 16). Data displayed as means and standard deviation (SD).

	Age (y)	**Body Mass Index**	**Weight (kg)**	**Blood glucose at screening (mg/dL)**
Males (n = 7)	32.3 (9.1)	24.4 (1.9)	81.0 (5.5)	89.6 (6.5)
Females (n = 9)	28.8 (8.1)	24.6 (4.6)	66.7 (15.2)	89.6 (7.5)
Total (n = 16)	30.3 (8.4)	24.4 (3.6)	72.9 (13.8)	89.6 (6.8)

To handle the unbalanced data set, a mixed methods linear model was constructed:

y ~ porridge preload*sweetness level + sex + period + (1|subject id)

Dunnett contrasts were performed to compare the barley porridge preload to the white rice control. The use of Dunnett contrasts as the main statistical analysis accommodates the loss in statistical power due to under-recruitment; barley porridge preloads were independently compared to the control white rice preload rather conducting comparisons across all preloads.

Discussion
Statistically significant findings were found for all three outcomes (blood glucose response, subjective appetite ratings and test lunch intake) under the unsweetened porridge preload conditions. Several of the barley porridge preloads were found to differ from the white rice control. This signifies large effect sizes; the trial is under-powered, but statistical differences were still found.

Publications, Handouts, Other Text & Web Products
We have a website (labs.wsu.edu/foodlab) that describes the study and helped with recruitment.

Outreach & Education Activities
Under supervision of Dr. Perrigue and Julianne Kellogg, one NEP Graduate student and four NEP Undergraduate students spent 3+ hours per week in the lab during Spring 2020, working on participant screening and assessment, meal preparation, data collection and entry, and other activities designed to increase knowledge and proficiency in a Human Research Laboratory setting. All students reported this was a strong learning experience.

Julianne Kellogg presented a poster on the research at the 2019 American Seed Trade Association's seed expo in Chicago. She won first place in the poster presentation competition. Additional presentations on the research topic and project:

- Kellogg, J. (2020). Biofortified barley. NEP 340 Food Science. WSU College of Medicine, Spokane, WA. October 23, 2020. Virtual presentation.
- Kellogg, J. (2020). Biofortification as a tool to tackle malnutrition: Experimentation in barley. Inland Northwest Research Symposium. WSU College of Medicine, Spokane, WA. March 27, 2020. Virtual presentation.
- Kellogg, J. (2021). Human Health from Soil to Society: Barley Beta-Glucan, Glycemic Control and Appetite. Inland Northwest Research Symposium, WSU College of Medicine, Spokane, WA. April 1, 2021, Virtual poster presentation.
- Kellogg, J. and M. Perrigue. (2021). Soil to Society: Barley Beta-glucan, Glycemic Control, and Appetite. GrainsWeek. WSU Food systems and Cascadia Grains, Olympia, WA. May 5, 2021. Virtual presentation.

Impacts

Short-Term: With the successful commencement of this trial, we have established the intended bridge between the Nutrition and Exercise Physiology Department and the Crop and Soil Sciences Department. Julianne Kellogg, a PhD student in Crop Science, was located at the WSU Spokane campus to work on this collaborative trial. This bridging of expertise has led to continued collaborative research.

Intermediate-Term: Increasing the accuracy of the NIR to estimate and rank β-glucan and protein content will speed up the nutritional phenotyping process in the WSU barley program.

Long-Term: Study findings will contribute to the growing body of evidence linking wholegrain barley β-glucan and positive health outcomes. Study findings have national and global implications. High fasting blood glucose and high body mass index are the leading risk factors for years lived with disability or injury in the U.S. [1]. Globally, one in three people is overweight or obese [2]. It is imperative that researchers continue to collaborate across agricultural and medical disciplines and work with food manufacturing stakeholders to improve the nutritive value of foods. This study demonstrates that increasing the β-glucan of barley can be an effective nutrition intervention to reduce post prandial glycemic response, appetite, and food intake.

Additional funding applied for/secured:

- Dept. of Nutrition and Exercise Physiology support for setting up the Food Lab
- Dr. Perrigue's startup fund used to support setting up the Food Lab

Graduate students funded:

- Julianne Kellogg, PhD candidate, Crop Science

Recommendations for future research: Continued research is needed on the impact of whole grain sources of β-glucan on post-prandial blood glucose response, appetite, and subsequent meal intake across different populations and using different whole grain barley products; such studies would to add to the body of evidence required to develop an approved health claim that can be used in marketing foods containing target levels of β-glucan.

Citations:

1. Heart Disease and Stroke Statistics—2021 Update Available online: https://www.ahajournals.org/doi/epub/10.1161/CIR.0000000000000950 (accessed on 8 June 2021).
2. Development Initiatives *2018 Global Nutrition Report: Shining a Light to Spur Action on Nutrition.*; Development Initiatives: Bristol, UK, 2018;