

Human Nutrition and Metabolism Research Communication

Carbohydrate Digestion in Humans from a β -Glucan-Enriched Barley Is Reduced^{1,2,3}

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ABSTRACT Obese and diabetic patients may benefit from foodstuffs that are poorly absorbed and/or digested at a slower rate. Prowashonupana (PW) is a cultivar of barley, whose grains are enriched in β -glucans, and thus may be less digestible than standard barley (barley cultivar (BZ) 594.35.e). To test this, both kinds of barley were grown in a chamber into which $^{13}\text{C}_2$ was injected. On two occasions, 10 healthy hydrogen (H_2)-producing adults consumed in random order one 35-g portion of each of the cooked, dehulled ^{13}C -enriched grains. CO_2 production was measured in a whole-body direct calorimeter, and H_2 and $^{13}\text{CO}_2$ were measured in breath at baseline and intermittently for 450 min. The percentage of the ^{13}C dose recovered in breath was calculated. Results were compared by repeated measures analysis of variance (ANOVA). The percentage of the ^{13}C dose oxidized was greater after BZ than after PW consumption ($P < 0.05$). The area under the curve for H_2 was greater after PW (mean \pm SD, 8658 \pm 6582) than after BZ (5178 \pm 4759) intake ($P < 0.05$), whereas there was no difference in CO_2 production. We conclude that absorption of PW is significantly lower than that of BZ, making the modified barley appropriate for obese and diabetic patients. *J. Nutr.* 132: 2593–2596, 2002.

KEY WORDS: • barley • stable isotope • β -glucan • obesity • diabetes

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Obese and diabetic patients may benefit from foodstuffs that are incompletely absorbed and/or digested at a slow rate. For the former group, foodstuffs that produce satiety but yield low energy may be an appropriate addition to the diet. For the latter, foodstuffs that elicit a moderate and sustained glycemic response may also be of benefit. In some cereals, food components such as β -glucans may lead to reductions in digestibility and carbohydrate use. β -glucans are viscous polysaccharides that, when included in a meal, result in a slower rate of carbohydrate and lipid absorption, which will modify the alimentary hormone and lipid responses. β -glucans, also referred to as β -D-glucans or mixed linkage β -glucans, are water-soluble polysaccharides present as dietary fiber in barley and oat grains (1). β -glucans are structural polysaccharides found in the cell walls of the bran layer and endosperm fractions of the whole seed (2). Structurally, β -glucans are linear chains of β -D-glucopyranosyl units in which $\sim 70\%$ of the units are linked (1 \rightarrow 4), but which also consist of β -D-cellobiosyl and β -D-cellobiosyl residues separated by (1 \rightarrow 3) linkages arranged in a random manner (3). The soluble nature of β -glucans, in conjunction with their chemical structure, helps to increase the viscosity of foods that contain them (1). Consumption of a barley-containing meal rich in β -glucans seems to stimulate reverse cholesterol transport, which may contribute to the cholesterol-lowering ability of barley (4). There is a cultivar of barley called prowashonupana (PW),⁵ whose grains contain elevated levels of β -glucans (5). The purpose of this study was to compare in healthy adults the energy use derived from a serving of PW with that derived from a serving of normal barley (barley cultivar 594.35.e (BZ)) using ^{13}C -labeled grains. Breath samples subsequently collected were analyzed for $^{13}\text{CO}_2$ as an indicator of the ^{13}C that was oxidized and hydrogen (H_2) as a measure of the carbohydrate that was not absorbed. When carbohydrate is incompletely absorbed by the small bowel it reaches the colon, where it is fermented by intestinal bacteria (6). A byproduct of this fermentation is H_2 , which is partially absorbed through the colonic mucosa, transported to the lungs, and exhaled in the breath, where it can be measured.

MATERIALS AND METHODS

Plant growth and ^{13}C labeling procedures

Two conventional cultivars of barley (*Hordeum vulgare* L.) were grown for grain production: cultivar PW (high β -glucan content; seeds kindly provided by ConAgra Oat Processing, Omaha, NE) and BZ 594.35.e (normal β -glucan content; seeds kindly provided by Dr. D. R. Clark, Western Plant Breeders, Bozeman, MT). Seeds were planted (25 per pot) in synthetic soil (Metro-Mix 360; Scotts-Sierra Horticultural Products, Marysville, OH) using 8.4-L plastic pots; planting density was 17 pots/m². Plants were maintained in a con-

⁵ Abbreviations used: ANOVA, analysis of variance; APE, atoms percent excess; BZ, barley cultivar BZ 594.35.e; PW, prowashonupana.

trolled environment chamber at 18°C and 50% relative humidity, and were illuminated continuously (24-h photoperiod) by a combination of incandescent and fluorescent lamps. Light intensity was adjusted to 390 μmol of photons per square meter per sec for the first 3 wk of growth, and 500 μmol of photons per square meter per sec for the remainder of growth. Both cultivars were grown in the same chamber at the same time. Plants were watered daily with a nutrient solution containing 1.2 mmol/L KNO_3 , 0.8 mmol/L $\text{Ca}(\text{NO}_3)_2$, 0.3 mmol/L KH_2PO_4 and 0.2 mmol/L MgSO_4 ; pots were watered to achieve full saturation of the soil.

Plants were pulse-labeled with $^{13}\text{CO}_2$ on three occasions during the period of grain fill; labelings occurred at 8, 12 and 16 d after 50% spike emergence. A total of 18.9 mmol of $^{13}\text{CO}_2$ was administered to each pot of plants over the course of the three labelings. For each labeling, groups of 12 or 13 pots were placed in a sealed Plexiglas enclosure (1.2 m^3) containing air-mixing fans, and connected in a closed loop with an infrared CO_2 gas analyzer (model 225-MK3; Analytical Development, Hertfordshire, UK). Labeling was conducted under a combination of natural lighting and metal halide lamps in a greenhouse.

Spikes with mature grains (~8% moisture) were harvested at ~6 wk after 50% spike emergence. Grains were removed from spikes and were dehulled by hand; hulls were separated from grains using an air stream.

Analysis of grain carbohydrates

A 10-g sample of labeled barley grains from each cultivar was dried and ground to a fine powder using a Wiley mill (Thomas Scientific, Philadelphia, PA) with a 60-mesh screen. Subsamples (50 mg) were used to determine total starch and total β -glucan content in the grains of each cultivar, using the techniques of Aman and Graham (3). Starch was assayed with a starch assay kit (SA-20; Sigma-Aldrich, St. Louis, MO) and β -glucans were assayed with a mixed-linkage β -glucan kit (Megazyme International, Wicklow, Ireland). Following the enzymatic assays, glucose monomers derived from the starch or β -glucan polymers were converted to penta-acetate derivatives (7). Glucose isotopomers were determined by selected ion monitoring of the methane positive ionization spectrum after gas chromatography/mass spectrometry (model 5989A; Hewlett Packard, Palo Alto, CA). Ions monitored were mass/charge 331–337.

Preparation of cooked barley

Servings (35 g) of the dehulled grains (~8% moisture) of each cultivar were weighed. Each serving was cooked 1 d before feeding using a rice steamer (Flavor Scenter Handy Steamer, model HS800; Black & Decker, Towson, MD) with deionized water. Barley and water were added to the cooking bowl in a 1:1 ratio (v/v); water also was added to the base of the steamer per the manufacturer's instructions. Total cooking time for the barley was 3 h (PW) or 2.5 h (BZ). After cooking, the barley was refrigerated in the cooking bowl overnight.

One serving of each cultivar was used for determination of total carbon content and ^{13}C enrichment using isotope ratio mass spectrometry techniques (see below). The total serving was dried and ground to a fine powder (0.25-mm sieve size) using a cutting mill (model SM1; Brinkmann Instruments, Westbury, NY) to homogenize each sample. Six subsamples of each cultivar were analyzed and their values were averaged to determine the dose of ^{13}C administered to each study subject.

Subjects and study design

The protocol was approved by the Baylor College of Medicine Institutional Review Board for Human Research and complied with the Helsinki Declaration as revised in 1983. Participants were 10 healthy adults (four male and six female; age range, 25–42 y; weight range, 52–77 kg) who had not received antibiotics for at least 15 d before the test. Subjects signed an informed consent and underwent a screening test to determine whether they were capable of producing H_2 following the ingestion of a nonabsorbable carbohydrate (lactulose).

For that purpose, subjects were instructed by a dietitian to ingest a low-fermentable dinner. After an overnight fast, they entered the Metabolic Research Unit of the Children's Nutrition Research Center. A breath sample was obtained by having each subject blow into a breath collection bag. Subjects then ingested a 25-g serving of cooked rice to which 15 g of lactulose syrup containing 10 g of a nonabsorbable carbohydrate (Duphalac; Solvay Pharmaceuticals, Marietta, GA) had been added. Breath samples were collected every 30 min during the ensuing 240 min. Subjects who mounted a H_2 response of 20 ppm or greater remained in the study and returned to the Metabolic Research Unit 7–10 d later. Subjects entered a whole-body indirect calorimeter where, under similar experimental conditions, 3–7 d apart, they ingested, in random order, a 35-g serving of one of the two barley cereals and 240 mL water. CO_2 production was measured continuously by the calorimeter. Breath samples were collected at baseline and intermittently for 450 min for measurement of H_2 and $^{13}\text{CO}_2$ abundance.

Calorimetry measurements

Oxygen consumption (O_2), production of carbon dioxide (CO_2) and the resultant respiratory quotient, defined as CO_2/O_2 , were measured continuously in a room calorimeter for 450 min. The operation, calibration and performance of the calorimeters have been described previously in detail (8). Each chamber has its own micro-processor-based gas analyzers for CO_2 (Ultramat 5E; Siemens, Karlsruhe, Germany) and O_2 (Oxymat 5E; Siemens) that enable continuous data collection, recorded at 1-min intervals. Thermal mass controllers (Sierra Instruments, Monterey, CA) regulate airflow through the chambers to maintain constant CO_2 concentration (0.45%) and gauge pressure (0.1 mm Hg). Errors from 24-h gas infusions averaged $0.34 \pm 1.24\%$ for O_2 and $0.11 \pm 0.98\%$ for CO_2 . Calorimeters were calibrated before each test. Calorimeter temperature and relative humidity were controlled between 23 and 25°C and 40–60%, respectively.

Breath sample analysis for $^{13}\text{CO}_2$ and H_2

Breath samples (Becton Dickinson, Franklin Lakes, NJ) were transferred from the bag and stored in air-tight Vacutainers and later analyzed by isotope ratio mass spectrometry using a RoboprepG attached to a 20:20 isotope ratio mass spectrometer (Europa Scientific, Franklin, OH) at the Children's Nutrition Research Center.

Samples were analyzed for H_2 content using a Quintron DP Microlyzer (Quintron, Menomonee Falls, WI) within 3 h of collection. Results are expressed as parts per million. Peak breath H_2 levels were calculated by subtracting from the highest breath H_2 level obtained during the test the lowest level. Peak breath H_2 levels of 10 ppm over baseline were considered indicative of carbohydrate malabsorption (9).

Calculations and data analysis

The percentage of the ^{13}C dose that was recovered in breath was calculated after correction for CO_2 production according to the following formulas: micromoles of CO_2 per min = (mean $\text{VCO}_2 \times 10^6)/22.4$; micromoles of ^{13}C excess = (micromoles of CO_2 per min \times APE enrichment)/100; and percent dose excess per min = (micromoles of ^{13}C excess $\times 10)/^{13}\text{C}$ dose administered, where 22.4 is the gas constant at standard temperature pressure to convert liters to micromoles and VCO_2 is the volume of CO_2 produced.

Statistical methods

The area under the breath H_2 curve was calculated using a computer program (Ms-Dos Q-basic; Microsoft, Redmond, CA). Results of the $^{13}\text{CO}_2$ in breath were compared by repeated measures ANOVA, with adjusted paired *t* tests to determine at which time points the means differed. Results are expressed as means \pm SD. Differences with a value of $P < 0.05$ were considered significant.

RESULTS

The barley labeling protocol generated grains with ^{13}C enrichments of 0.6367 atoms percent excess (APE) (PW) or 0.5167 APE (BZ). As anticipated for these cultivars, PW grains were higher in β -glucan (17.7 g/100 g dry grain) and lower in starch (25.9 g/100 g dry grain), relative to β -glucan (5.3 g/100 g dry grain) or starch (58.5 g/100 g dry grain) in BZ. Although carbohydrate compositions differed (see below), there were no differences in the glucose isotopomer profiles derived from the starch of β -glucan fractions for either barley. For either labeled polymer, mean glucose isotopomer percentages were as follows: M, 81.9%; M + 1, 14%; M + 2, 2.9%; M + 3, 0.8%. Thus, there were no differences in the ^{13}C enrichments of the starch of β -glucan fractions between the two cultivars. The difference between the two kinds of barley was accounted for by soluble fiber.

The modified barley was well tolerated. There was no difference in CO_2 production or oxygen consumption following the ingestion of the two test cereals. The overall percent dose of ^{13}C oxidized was higher after BZ than after PW intake ($P < 0.05$) (Fig. 1). Breath $^{13}\text{CO}_2$ in samples obtained within 120 min of the ingestion of the cereals was significantly greater after consumption of BZ than of PW (Fig. 1). The area under the curve for H_2 was higher ($P < 0.05$) after PW intake (8658 ± 6582) than after BZ (5178 ± 4759). The breath H_2 levels following PW and BZ intakes differed in the samples obtained between 120 and 210 min (Fig. 2).

DISCUSSION

Dietary management of type II diabetes mellitus is geared toward improvement of glucose and lipid control. This includes a low-fat, high-carbohydrate diet, particularly one based on cereals. However, cereal products are rapidly digested and absorbed and therefore tend to have a high glycemic index. For the dietary management of obesity, foods that have a familiar taste, produce prolonged satiety and are incompletely absorbed are ideal. In some cereals, food components such as β -glucans may reduce digestibility and carbohydrate utilization. PW is a cultivar of barley that is the result of selected breeding, yielding grains containing low starch, high protein and elevated levels of β -glucans. When cereals such as PW are consumed with a meal, once the bolus reaches the small intestine the viscosity of the meal is increased. This high

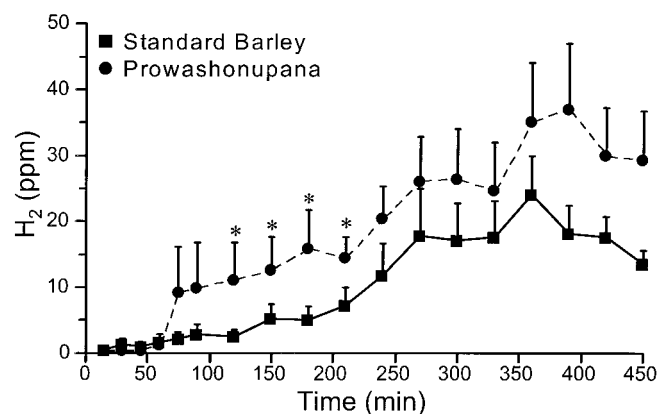


FIGURE 2 Peak breath H_2 levels by humans after consumption of standard barley (BZ) and prowashonupana (PW). Values are means \pm SD; $n = 10$. *, Means differed ($P < 0.05$).

viscosity delays absorption. A 50% reduction in the glycemic peak has been achieved with a concentration of 10% β -glucans in a cereal (10,11). Intrinsic labeling of plants with stable isotopes constitutes a safe way to study absorption and utilization of nutrients (12). The labeling of all organic molecules can be achieved through the introduction of $^{13}\text{CO}_2$ to the plant because carbon fixed in the photosynthetic processes is ultimately passed on to all biosynthetic pathways (13). The primary requirement of a $^{13}\text{CO}_2$ labeling system is a closed, relatively gas-tight chamber to house the plants and to isolate the enriched atmosphere. In the past, we have used ^{13}C -enriched rice to determine its absorption in infants with diarrhea (14).

In the present study, we used two indicators: one of oxidation (digestion and utilization) and one of malabsorption. For the former, we measured utilization of C from barley by measuring $^{13}\text{CO}_2$ excreted in breath. For the latter, we measured breath H_2 , an indicator of malabsorbed carbohydrate. Breath $^{13}\text{CO}_2$ in samples obtained in the first 120 min following ingestion of the cereals differed, indicating a difference in the rate of utilization by the subjects. As time elapsed, $^{13}\text{CO}_2$ generated in the colon by fermentation of malabsorbed barley and recirculation of ^{13}C through the bicarbonate pool resulted in $^{13}\text{CO}_2$ outputs that were not different from one another (15). The breath H_2 levels after PW and BZ ingestion were significantly different in the samples obtained between 120 and 210 min. Before that time, the difference in the amount of the two types of barley that had reached the colon was not large enough to be detected. These results reflect the approximate time it takes for the cereal to arrive in the colon and be fermented by colonic bacteria. The combination of the two tests used in this study indicates lower oxidation of PW due to decreased absorption of this type of barley compared with BZ. De Vries et al. (16) used barley groats to determine the mouth to cecum transit times. They found that H_2 was detected in breath in all subjects by 8 h post-ingestion and in some at 2 h and 45 min. In our study H_2 was detected in breath at much earlier times. This difference can be attributed to the amount of cereal ingested (much lower in our study) and/or the fact that we used cooked barley whereas De Vries et al. (16) used barley just softened in water. Behall et al. (17), using a serving size of oats similar to the one administered by De Vries et al. (16), did not observe a difference between cooked and uncooked cereal in the amount of H_2 detected in breath but did not report the mouth to cecum transit time (17).

A previous study demonstrated that ingestion of PW re-

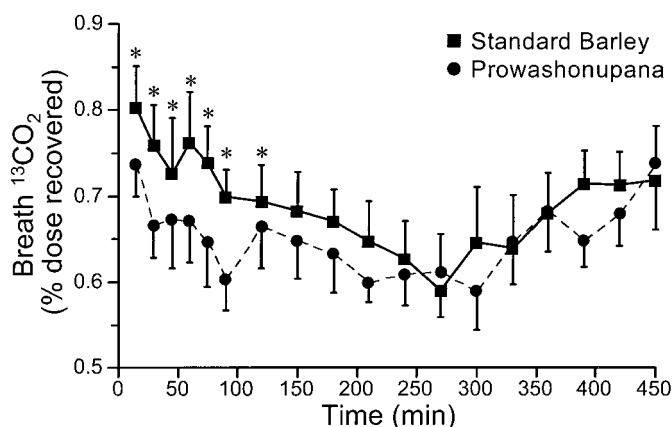


FIGURE 1 Percent dose of ^{13}C oxidized by humans after consumption of standard barley (BZ) and prowashonupana (PW). Values are means \pm SD; $n = 10$. *, Means differed ($P < 0.05$).

sulted in a significantly lower postprandial glycemic response compared with that of Sustacal (Ross Laboratories, Columbus, OH) or oatmeal (18). Our results support the conclusion of Battilana et al. (19), who stated that the lowered postprandial glycemic response following a meal containing β -glucans is related not to changes in carbohydrate or lipid metabolism but to delayed or decreased absorption.

In summary, PW is less well absorbed and used than BZ. The modified barley thus could serve as a nutritionally appropriate food item for patients with diabetes or obesity.

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