Carbohydrate Digestion in Humans from a β-Glucan-Enriched Barley Is Reduced1,2,3

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Carlos H. Lifschitz,4 Michael A. Grusak and Nancy F. Butte
U.S. Department of Agriculture/ARS Children’s Nutrition Research Center and Texas Children’s Hospital, Department of Pediatrics, Baylor College of Medicine, Houston, TX 77030

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 $^{13}$CO$_2$ was injected. On two occasions, 594.35.e). To test this, both kinds of barley were grown in a whole-body direct calorimeter, and H$_2$ and $^{13}$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 ± so, 8658 ± 6582) than after BZ (5178 ± 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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4 To whom correspondence should be addressed. E-mail: carlosl@bcm.tmc.edu.

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 watersoluble 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 ~70% of the units are linked (1→4), but which also consist of β-D-cellotriosyl and β-D-cellotetraosyl residues separated by (1→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), 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 $^{13}$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/m$^2$. Plants were maintained in a con-
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 380 μmol 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 KNO₃, 0.8 mmol/L Ca(NO₃)₂, 0.3 mmol/L KH₂PO₄ and 0.2 mmol/L MgSO₄; pots were watered to achieve full saturation of the soil.

Plants were pulse-labeled with ¹³CO₂ 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 ¹³CO₂ 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 m²) containing air-mixing fans, and connected in a closed loop with an infrared CO₂ 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.

Spike 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 (30 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 isotope determiners were selected by ison monitoring of the methane positive ionization spectrum after gas chromatography/mass spectrometry (model 5989A; Hewlett Packard, Philadelphia, PA) with a 60-mesh screen. Subsamples (50 mg) were infused by the calorimeter. Breath samples were collected every 30 min during the ensuing 240 min. Subjects who maintained a H₂ response of 20 ppm or greater remained in the study and returned to the Metabolic Research Unit 7–10 d later. Subjects were enrolled in a whole-body indirect calorimeter where, under similar experimental conditions, breath samples were collected to determine total carbon dioxide production and measured continuously by the calorimeter. Breath samples were collected at baseline and intermittently for 450 min for measurement of H₂ and ¹³CO₂ abundance.

### Calorimetry measurements

Oxygen consumption (O₂), production of carbon dioxide (CO₂) and the resultant respiratory quotient, defined as CO₂/O₂, 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 microprocessor-based gas analyzers for CO₂ (Ultramat 5E; Siemens, Karlshorst, Germany) and O₂ (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 CO₂ concentration (0.45%) and gauge pressure (0.1 mm Hg). Errors from 24-h gas infusions averaged 0.34 ± 1.24% for O₂ and 0.11 ± 0.98% for CO₂. 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 ¹³CO₂ and H₂

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 H₂ content using a Quintron DP Microlyzer (Quintron, Menomonee Falls, WI) within 3 h of collection. Results are expressed as parts per million. Peak breath H₂ levels were calculated by subtracting from the highest breath H₂ level obtained during the test the lowest level. Peak breath H₂ levels of 10 ppm over baseline were considered indicative of carbohydrate malabsorption [9].

### Calculations and data analysis

The percentage of the ¹³C dose that was recovered in breath was calculated after correction for CO₂ production according to the following formulas: micromoles of CO₂ per min = (mean VCO₂ × 10⁶)/22.4; micromoles of ¹³C excess = (micromoles of CO₂ per min × APE enrichment)/100; and percent dose excess per min = (micromoles of ¹³C excess × 10)/¹³C dose administered, where 22.4 is the gas constant at standard temperature pressure to convert liters to micromoles and VCO₂ is the volume of CO₂ produced.

### Statistical methods

The area under the breath H₂ curve was calculated using a computer program (Ms-Dos Q Basic; Microsoft, Redmond, CA). Results of the ¹³CO₂ 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 ± 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 $\beta$-glucan (17.7 g/100 g dry grain) and lower in starch (25.9 g/100 g dry grain), relative to $\beta$-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 $\beta$-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 $\beta$-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 $^{13}$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. 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 $^{13}$CO$_2$ excreted in breath. For the latter, we measured breath H$_2$, an indicator of malabsorbed carbohydrate. Breath $^{13}$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, $^{13}$CO$_2$ generated in the colon by fermentation of malabsorbed barley and recirculation of $^{13}$C through the bicarbonate pool resulted in $^{13}$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 postingestion 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](https://example.com/figure1.png)

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

![Figure 2](https://example.com/figure2.png)

**FIGURE 2** Peak breath H$_2$ levels by humans after consumption of standard barley (BZ) and prowashonupana (PW). Values are means ± so; 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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LITERATURE CITED