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High-molecular-weight barley β-glucan in chapatis (unleavened Indian flatbread) lowers glycemic index

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Abstract

Food products incorporated with soluble dietary fiber β-glucan have shown varying effects on postprandial glycemia. The objective of the present study was to test the hypothesis that a food product fortified with barley β-glucan and subjected to minimum processing and mild cooking might be effective in lowering glycemic response. In a randomized, single-blind, controlled crossover trial, 8 healthy human subjects (3 men, 5 women; aged 26-50 years; body mass index, <30 kg/m^2) consumed unleavened Indian flatbreads called chapatis containing high-molecular-weight barley β-glucan at doses of 0, 2, 4, 6, and 8 g on different occasions. Capillary blood samples were collected at 0, 15, 30, 45, 60, 90, and 120 minutes after consuming the chapatis. The incremental area under the glucose curve values for all the 5 different types of chapatis were significantly low ($P < .001$) compared with reference food glucose. The incremental area under the glucose curve of chapatis containing 4 and 8 g β-glucan were significantly lower than control chapatis ($P < .05$). Postprandial blood glucose was significantly reduced at 45 minutes by chapatis containing 4 g ($P < .05$) and 8 g β-glucan ($P < .01$) and at 60 minutes by chapatis with 8 g β-glucan ($P < .01$). The glycemic index (GI) values of chapatis with 4 and 8 g β-glucan were 43% to 47% lower (GI, 30 and 29, respectively) compared with chapatis without β-glucan (GI, 54). We conclude that barley β-glucan significantly reduces GI of chapatis, particularly at doses of 4 and 8 g per serving.

Keywords: Area under the curve; Barley β-glucan; Blood Glucose; Chapatis; Glycemic index; Human; Randomized crossover trial

Abbreviations: ANOVA, analysis of variance; FAO/WHO, Food and Agriculture Organization/World Health Organization; GI, glycemic index; GR, glycemic response; HMW, high molecular weight; IAUC, incremental area under the glucose curve.

1. Introduction

Changes in lifestyle including dietary factors and lack of exercise have resulted in a number of health-related problems in developing and developed countries. There has been growing concern regarding the increasing prevalence of obesity worldwide among both children and adults. Obesity is an important risk factor in contributing to the development of type 2 diabetes and cardiovascular diseases. The risk of developing type 2 diabetes and heart diseases is reduced by the intake of whole-grain diets with high dietary fiber content and low glycemic index (GI) [1]. The concept of GI was first introduced in 1981 as a means for identifying and classifying carbohydrate-rich foods based on their ability to raise postprandial blood glucose levels [2]. A low or attenuated glycemic response (GR) is beneficial in both healthy and diabetic people. In the past few years, researchers have focused on developing foods with low GI

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and achieved favorable results by incorporating dietary fiber from cereals in various foods such as breads, pasta, and beverages [3-7].

Soluble dietary fiber is present in several forms in cereals including β-glucans in oats, barley, rye, and wheat. They are linear homopolysaccharides of glucose residues connected via a mixture of β-(1 → 3) and β-(1 → 4) linkages, resulting in tricellulosyl and tetracellulosyl units linked by single (1 → 3) linkages [8]. The β-glucan content in barley ranges from 2.5% to 11.3%, whereas oats (2.2%-7.8%), rye (1.2%-2.0%), and wheat (0.4%-1.4%) have lower levels [9]. Historically, barley was one of the most ancient crop plants grown mostly for human consumption. However, consumption of barley decreased considerably in modern times restricting its use in brewing and as animal feed. It is anticipated that recent findings relating to the health benefits of barley would stimulate food industries and consumers to include barley in their daily diet. The US Food and Drug Administration has allowed whole-grain barley products that can supply β-glucan at levels of 0.75 g per serving or 3 g per day to carry a claim that they reduce the risk of coronary heart disease (Food and Drug Administration News Release, 2005).

The efficacy of β-glucans as potential functional food ingredients may be related to their structure, molecular weight, and rheological characteristics, which, in turn, can be affected by cooking methods. Food processing methods have been found to degrade β-glucan, leading to loss of its functional properties. In a study using oat products, the deleterious effects of processing were more pronounced after baking, fermentation, frying, and pasta and pancake preparation [10]. The molecular weight of β-glucan was also found to be low in yeast-leavened barley bread [11]. A number of studies have evaluated the effect of barley β-glucans in traditional food products such as bread, pasta, cereal flakes, and others on postprandial glycemia in both healthy [6,7,12-14] and overweight subjects [15].

Chapatis are a form of unleavened flatbreads of Indian/Eastern origin. They form an integral part of Indian diet, especially for those who have type 2 diabetes for whom white rice is considered less desirable because of its high GI. Chapatis and other flatbreads are popular in Europe also where they form a part of daily diet among members of ethnic minority groups who follow traditional dietary patterns. Chapatis are made from whole-wheat flour and cooked on hot flat open griddles. They can also be prepared by substituting wheat flour with other cereal or legume flours at different levels. This could result in either improvement or deterioration of texture and taste of chapatis. Incorporation of barley flour at proportions above 10% has resulted in inferior dough structure and lower volume in chapatis made of wheat flour [16]. The color and appearance of chapatis were found to be good with substitution of wheat flour with up to 30% hulless barley flour, whereas flavor and texture were acceptable even at 40% substitution levels [17]. The extensibility of cooked chapatis was found to increase at 20% substitution of wheat flour with barley flour [18].

A diet rich in functional food components such as barley β-glucans incorporated in some of the staple food items can play an important role in reducing the prevalence of diabetes and coronary heart diseases. Previous studies have shown varying responses to food products with barley β-glucans depending on their molecular weight and extent of cooking/processing (10-14). We hypothesized that high-molecular-weight (HMW) barley β-glucan added to a food product subjected to mild cooking would be effective in lowering postprandial glycemia. Therefore, objectives of the present study were as follows:

1. To test the GR to chapatis with added barley β-glucan in healthy subjects
2. To evaluate whether cooking affects the GI lowering potential of barley β-glucan added to whole-wheat flour
3. To study the dose-response effect of barley β-glucan incorporated in chapatis in lowering postprandial blood glucose

2. Methods and materials

2.1. Subjects

Eight healthy human subjects (3 men, 5 women) from among the staff and students of Oxford Brookes University were recruited to participate in the study. The participants were between 26 and 50 years old, had a body mass index less than 30 kg/m^2, and were not involved in any heavy physical activity other than normal day-to-day household and office work. They were required to complete a health questionnaire to check against any health conditions that might affect the study such as abnormal blood glucose levels or any medical conditions or intake of drugs that might affect glucose metabolism, absorption, and appetite. Participants were given complete details of the study protocol and were free to ask any questions or withdraw from the study at any time. Written informed consent was obtained from all the subjects before participating in the study. Ethical approval for the study was obtained from the University Research and Ethics Committee at Oxford Brookes University.

Anthropometric measurements were made for all the subjects, using standardized methods before the beginning of the study. Height was recorded to the nearest centimeter using Stadiometer (Seca Ltd, Birmingham, UK), with the subjects standing erect without shoes. Body weight was recorded using the Tanita BC- 418 MA (Tanita UK Ltd, Yiewsley, UK), with the subjects wearing light clothing and no shoes. Body mass index was calculated with the standard formula weight (in kilograms)/height (in meters^2). Table 1 shows the anthropometric measurements of the subjects.

2.2. Study design

The protocol used to measure GR and calculate GI of test meals was adapted from previous studies [19,20] in

Table 1
Baseline characteristics of the subjects who completed the study

	Measurement
Age (y)	38 ± 11
Height (cm)	165 ± 0.1
Weight (kg)	63.6 ± 13.6
BMI [a] (kg/m^2)	23.2 ± 3.5

Values are means ± SD. BMI indicates body mass index.
[a] BMI was calculated as weight/height^2.

agreement with the procedures recommended by Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1998) [21]. All the foods were tested after a 12-hour overnight fast. As the subjects arrived in the morning, they were required to complete a small questionnaire regarding last meal eaten, amount of alcohol and coffee consumed, exercise duration, and level of stress. In a randomized, single-blind, controlled crossover trial, the subjects consumed glucose (reference food) or test chapatis (with 0, 2, 4, 6, and 8 g of barley β-glucan) equivalent to 50 g carbohydrate as breakfast. Available carbohydrate in each test meal was calculated using the FAO/WHO procedure (total carbohydrate minus dietary fiber) according to the nutrition information available from the manufacturers of chapati flour and barley β-glucan.

The reference food—50 g glucose—was tested on 3 separate occasions to check the variation in blood glucose levels in each subject. The reference food and test chapatis were served with 200 mL water, and the subjects were required to eat at a comfortable pace within 10 to12 minutes. There was no further liquid intake during the test period of 2 hours. A minimum of 1-day gap was maintained between the test meals to minimize any carryover effect from one meal to the other.

2.3. Test meals

The reference food glucose (dextrose monohydrate) was from Lloyds Pharmacy Limited, Coventry, UK. Chapatis were made using Pillsbury whole-wheat flour (General Mills India Pvt. Ltd, Mumbai, India) purchased from the local supermarket. Barley balance (Polycell Technologies, Crookston, Minn), used as the source of β-glucan, was provided by DKSH, Wimbledon, London. Barley balance (with 26% β-glucan) was weighed out in such a way that the final formulation contained 2, 4, 6, and 8 g β-glucan equivalent to 2%, 4.1%, 6%, and 7.8%, respectively. Chapati dough with 0, 2, 4, 6, and 8 g β-glucan was prepared by mixing the whole-wheat flour with barley balance and water (75% vol/wt) and kneading for 5 minutes. The dough was divided into 4 portions of equal size and rolled into chapatis using a traditional rolling pin. Rolled chapatis were cooked for 2 minutes on a hot flat griddle by turning each side after 1 minute. Table 2 shows the energy and nutrient composition of the test chapatis in a 50-g available carbohydrate portion.

2.4. Blood glucose measurements

Fasting blood glucose measurement was made at −5 and 0 minutes before consumption of the test meal, and the means of both the values were used as the baseline blood glucose value. Further blood samples were taken after 15, 30, 45, 60, 90, and 120 minutes after the subjects started eating the test meal. Subjects were encouraged to warm their hands to increase blood flow before taking the blood sample. Capillary blood glucose was analyzed in finger-prick blood samples obtained using the Unistik 3-single-use lancing device (Owen Mumford, Woodstock, UK). After discarding the initial 2 blood drops, the third drop was drawn into Hemocue Glucose 201 microcuvette by capillary action. Glucose was measured by placing the microcuvettes in Hemocue Glucose 201+ blood glucose analyzer (Hemocue Ltd, Dronfield, UK).

2.5. Calculation of GR and GI

The incremental area under the glucose curve (IAUC) for reference food and test chapatis was calculated geometrically using the trapezoidal rule [19,21]. All areas below the baseline were excluded from the calculations. The blood glucose response and IAUC of the reference food were reported as the mean of the 3 tests carried out for each subject. The GR for each test chapati was determined by expressing the IAUC of the test chapati for each subject as a percentage of the mean IAUC of the reference food consumed by the same subject. Glycemic index was calculated from the IAUCs with each subject acting as his or her own reference.

2.6. Statistical analyses

The results are expressed as means ± SD. Statistical analysis was performed using Statistical Package for the Social Sciences (version 17.0; SPSS, Chicago, Ill). The intraindividual variations of the 3 reference glucose tests were assessed by determining the percent coefficient of variation (CV% = 100 x SD / mean). Paired *t* test was used to assess the differences between IAUCs [22]. Significances

Table 2
Energy and nutrient composition of the test chapatis containing 0, 2, 4, 6, and 8 g β-glucan per 50 g available carbohydrate portion

	Chapati type [a]				
	1	2	3	4	5
Portion size (g)	134	139	142	147	151
Total carbohydrate (g)	59.1	62.5	63.2	65.2	67
Protein (g)	10.9	11.9	12.4	13.1	13.8
Fat (g)	1.5	1.64	1.7	1.7	1.8
Total fiber (g)	9.1	11.3	13.1	15.2	17.2
β-Glucan (g)	0	2	4	6	8
Energy (kJ)	1232	1307	1328	1375	1420

Calculations were based on the nutrition information supplied by the manufacturers of wheat flour and β-glucan–enriched barley flour.
[a] Chapati type (amount of β-glucan): 1 (0 g), 2 (2 g), 3 (4 g), 4 (6 g), and 5 (8 g).

between the differences in blood glucose values were evaluated by 1-way analysis of variance (ANOVA), followed by Tukey multiple comparisons test. Statistical significance was set at $P < .05$.

3. Results

The FAO/WHO recommends that tests should be conducted in 6 or more subjects to determine the GI of a food [21]. The results presented are from 8 human subjects who completed the study. The subjects did not report any change in palatability of chapatis after β-glucan supplementation. It was also observed that during cooking, β-glucan containing chapatis puffed up better than the whole-wheat ones. The GR to all the chapatis added with 2, 4, 6, and 8 g of HMW barley β-glucan were significantly lower when compared with the reference food, glucose. This is in agreement with results obtained using β-glucan–rich barley products [15]. When compared with the control chapatis, the GI values for β-glucan–supplemented chapatis were significantly lower at doses of 4 and 8 g β-glucan per serving.

3.1. Peak rise in blood glucose

Fig. 1 shows the incremental blood glucose response curves for the reference food, glucose, and different types of chapatis with 0, 2, 4, 6, and 8 g barley β-glucan. When compared with reference glucose, delta glucose values were significantly less for all the chapati types at 15, 30, and 45 minutes ($P < .001$). The peak blood glucose value was reached at 30 minutes for the reference food glucose and at 45 minutes for chapatti having 0, 2, and 6 g β-glucan. However, with chapati having 4 g β-glucan, there was a delayed rise in blood glucose reaching the peak value at 60 minutes. In the case of chapati with 8 g β-glucan, the peak blood glucose level was reached relatively early at 30 minutes but was maintained almost at the same level until 60 minutes. With all the treatments, the blood glucose levels rapidly dipped after 60 minutes, except for chapati with 8 g β-glucan where it declined slowly.

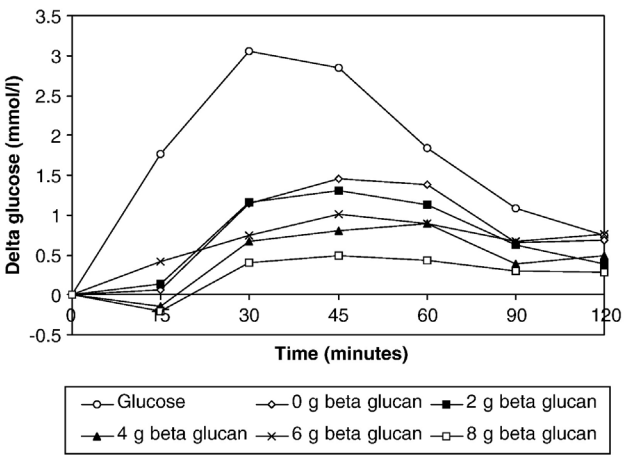

Fig. 1. Change in blood glucose (means) in healthy subjects after consuming glucose and chapatis with 0, 2, 4, 6, and 8 g of barley β-glucan. Statistical significance was evaluated by 1-way ANOVA. Delta glucose values were significantly less than reference glucose for all the chapati types at 15, 30, and 45 minutes ($P < .001$). Values for chapatis with 4 g β-glucan at 45 minutes ($P < .05$) and chapatis with 8 g β-glucan at 45 and 60 minutes ($P < .01$) are statistically significant compared with chapatis with no β-glucan.

There was no significant difference between the blood glucose response to control chapati and chapati containing 2 g β-glucan. Chapati with 4 g β-glucan showed significant reduction in postprandial blood glucose levels after 45 minutes of consumption ($P < .05$). The reduction in postprandial blood glucose was significant with chapati containing 8 g β-glucan at 45 and 60 minutes ($P < .01$). Another interesting observation was that with all the treatments, there was a delayed rise in blood glucose, especially with 4 and 8 g β-glucan where the blood glucose levels went below the baseline in the first 15 minutes before increasing moderately again (Fig. 1). An exception in this trend was observed with 6 g β-glucan, in which there was a steady increase in blood glucose levels similar to the pattern obtained with reference food, glucose. Interestingly, all the subjects showed the same trend with this meal type. The interindividual variation in GI was also high for this meal, thereby resulting in high SD values compared with other test meals.

3.2. Changes in IAUC values

Table 3 shows the IAUC values for chapatis with different levels of β-glucans. The IAUC values for all the 5 different chapatis (with and without β-glucan) were significantly lower compared with reference food, glucose ($P < .001$). The percent reductions in IAUC for 4 and 8 g β-glucan were 68% to 71% (±10) in comparison with the reference food, glucose, and 40 ± 13% to 47 ± 10% when compared with chapati without β-glucan ($P < .05$). There was no significant difference between IAUC values for control chapati and chapati containing 2 g β-glucan. The IAUC value for chapatis with 6 g β-glucan showed an insignificant 16 ± 12% reduction from control chapatis and was found to be intermediate between chapatis with 2 and 4 g β-glucan.

3.3. GI of test chapatis

Fig. 2 shows the GI values of chapatis with β-glucan at 0, 2, 4, 6, and 8 g/50 g available carbohydrate. The mean GI value of control chapati and chapati with 2 g β-glucan was 54, showing no significant difference between them. In correlation with the IAUC values, the GI values for chapatis with 4 and 8 g β-glucan were 43% to 47% less than the control chapatis made of whole-wheat flour. The GI of chapatis with 6 g β-glucan was found to be reduced insignificantly by 17% only.

4. Discussion

The present study shows reduction in GR to whole-wheat chapatis using a barley β-glucan of molecular weight ranging

Table 3
Postprandial IAUC values after consumption of glucose and chapatis containing 0, 2, 4, 6, and 8 g of HMW barley β-glucan

Test food	IAUC 0-120 min	% Reduction in IAUC compared with chapati without β-glucan	% Reduction in IAUC compared with glucose
Glucose	186.4 ± 38.7[a]		
Chapati with 0 g β-glucan	102.2 ± 43[b]		46 ± 17 [1]
Chapati with 2 g β-glucan	100.8 ± 21.8[b]		45 ± 13 [1]
Chapati with 4 g β-glucan	61.3 ± 30.3[cd]	40 ± 13 [2]	68 ± 10 [1]
Chapati with 6 g β-glucan	85.9 ± 39[bd]	16 ± 12 [3]	53 ± 19 [1]
Chapati with 8 g β-glucan	53.9 ± 22.8[cd]	47 ± 10 [2]	71 ± 10 [1]

Values are expressed as means ± SD for 8 healthy human subjects. The IAUC values were calculated using the trapezoidal rule and their differences assessed by paired *t* test. Statistical significance was evaluated by 1-way ANOVA. Values with different superscript letters in a column are significantly different.

[1] Values significantly lower than reference food glucose, $P < .001$.

[2] Values significantly lower than chapati without β-glucan, $P < .05$.

[3] Value not significantly different from chapati without β-glucan. $P > .05$.

form 650 000 to 700 000 obtained by dry milling and separation process without involving any chemicals (personal communication with the manufacturer). Results showed a mean GI value of 54 for chapatis made of whole-wheat flour. This is in agreement with the classification of whole-wheat chapatis as food with medium GI and their reported GI value (52 ± 4) [23]. Efforts were made in this study to further lower the GI of whole-wheat chapatis by adding β-glucan–enriched barley flour. Although some studies have evaluated the sensory and texture attributes of chapatis by addition of various flours [17,18,24,25], there are very few reports on metabolic responses to barley chapatis. When healthy subjects consumed chapatis made of 100 g barley flour everyday for 4 weeks [26] and did a Meal Tolerance Test using 50 g white bread at the beginning and end of each dietary period, their IAUC (mg dL^{-1} 3 h^{-1}) values were decreased from 107.9 ± 54.8 at 0 week to 91.5 ± 30.8 at 4 weeks. The authors attributed this change to water-soluble β-glucan present in barley flour. When GI of maize, bajra, and barley were compared, the GI of barley chapatis was found to be 68.7 in healthy subjects and 53.4 in diabetic subjects [27].

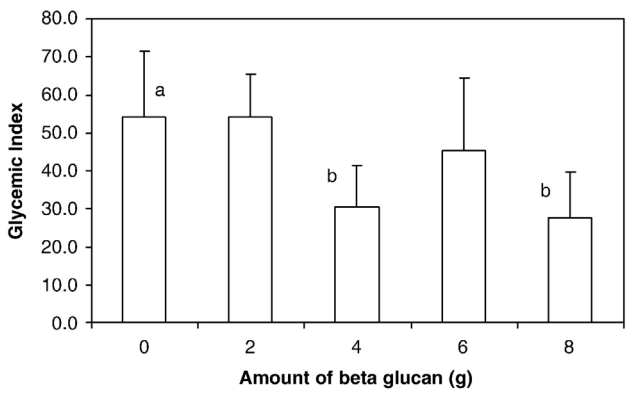

Fig. 2. Glycemic index values (means ± SD) of chapatis containing 0, 2, 4, 6, and 8 g of barley β-glucan. Statistical significance was evaluated by 1-way ANOVA. Bars having different letters are significantly different ($P < .01$ for 4 g of β-glucan and $P < .05$ for 8 g of β-glucan).

Molecular weight and viscosity are the 2 important characteristics that determine the physiological effects of β-glucans [28]. The functional properties of β-glucans have always been attributed to their ability to delay carbohydrate digestion and absorption from the gut by increasing the viscosity of the stomach and intestinal contents [8] and forming a protective layer incorporating readily digestible carbohydrates [29]. Processing and cooking have been shown to significantly alter the above-mentioned properties and the resultant functional benefits of β-glucans [7,30-32]. Hence, in the present study, chapatti—a food product that needs mild cooking conditions (contact heat in a griddle)—was used to test the effect of added β-glucan on its GI. Although there was a significant reduction in the GI of chapatis by adding 4 and 8 g β-glucan, there was no dose-dependent difference between the 2 test chapatis. Hence, it could be postulated that 4 g is the ideal dose of β-glucan required to reduce GI of chapattis, and higher doses would not bring about any further additional reduction in GI.

Various studies have assessed different food products incorporated with β-glucan and its effect on glucose and insulin responses as well as blood pressure, lipids, and cholesterol levels and satiety [33-37]. Addition of HMW barley β-glucan did not change GI of bread in hypercholesterolemic subjects [32], whereas significant GI reduction was observed using bread with 6% and 12% β-glucan [6,14]. In the present study, a similar reduction in GI (43%) of chapatis was achieved using much lower concentration of β-glucan (4 g). The above results indicate that the amount of β-glucan required to reduce the GI of different foodstuffs might not be the same, and using a known concentration of β-glucan might produce dissimilar effects in different food products.

There are limited reports on the dose-response effect of barley β-glucan. In the present study, addition of 4 g of β-glucan in chapatis resulted in significant GI reduction, but there was no further significant reduction in GI with 6 and 8 g β-glucan. This is in contrast to the results obtained in a previous study in which 0, 2, 4, and 8 g of barley β-glucan in 75 g glucose drink reduced postprandial glucose and insulin levels in a dose-dependent manner [38]. It is possible that the

viscosity induced by increasing doses of β-glucan in a drink is more linear with less interference from other factors involved in solid food digestion such as mastication, rate of digestion, and degree of absorption. Nonlinear GR to increasing doses of oat β-glucan was reported by Makelainen et al [29], who compared drinks containing 2, 4, and 6 g of oat β-glucan and found that the product with 6 g β-glucan had a GI value higher than the product with 2 g β-glucan but lower than the product with 4 g β-glucan. The authors attributed this to the amount of extractable β-glucan present, which was low in the product containing 6 g β-glucan but high in the product containing 4 g β-glucan. Such changes in amount of extractable β-glucan could be responsible for the nonlinear effect of β-glucan on GI of chapatis too. However, the study did not investigate the effect of higher levels of oat β-glucan above 6 g.

Addition of barley β-glucan could be a highly effective method for lowering the GI of chapatis, which is widely consumed by type 2 diabetes patients living in India, South Asia, and the expatriate community of Indian origin living worldwide. The amount of β-glucan required to bring about a remarkable reduction in GI is only 4 g, which is very small, compared with that reported in literature [12,15]. The IAUC values for chapatis containing 4 and 8 g β-glucan were reduced by 68% to 71% (±10) when compared with reference food, glucose. Such remarkable reduction has never been reported using any food product fortified with β-glucan. However, this study had a few limitations, which included the inability to monitor changes in gastrointestinal viscosity and the effect of chewing on β-glucan–enriched chapatis. The measurement of above-mentioned factors might have answered the lack of linear dose-dependent effect of higher doses of β-glucan above 4 g on GI. Lack of linear response to added β-glucan at higher doses (6 and 8 g) in this study could have been due to their inability to increase gastrointestinal viscosity in a linear manner. Further studies are in progress to find out whether viscosity induced during in vitro digestion of chapatis containing β-glucan is linear and dose dependent. Also, we should have used doses above 8 g of β-glucan to find out whether further GI reduction could be achieved.

In conclusion, this study showed that supplementation of whole-wheat flour with HMW β-glucan could be an efficient way of making palatable chapatis for diabetic patients. Supplementation at a dose of 4 g β-glucan per serving was highly significant and critical in reducing GI with no further dose-dependent reduction at higher levels of β-glucan. This indicates the importance of continuous intake of soluble dietary fiber-like barley β-glucan at moderate levels and the role it can play in reducing the risk of type 2 diabetes worldwide by incorporating barley β-glucan into some of the staple diets.

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