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Physicochemical Properties of β-Glucan in Differently Processed Oat Foods Influence Glycemic Response

Alejandra Regand,[†] Susan M. Tosh,*[,†] Thomas M. S. Wolever,[‡] and Peter J. Wood[†]

[†]Agriculture and Agri-food Canada, Food Research Program, 93 Stone Road, West Guelph, Ontario N1G 5C9, Canada, and [‡]Glycemic Index Laboratories, 36 Lombard Street, Suite 100, Toronto ON M5C 2X3, Canada

To assess the effect of food processing on the capacity of oat β-glucan to attenuate postprandial glycemia, isocaloric crisp bread, granola, porridge, and pasta containing 4 g of β-glucan as well as control products with low β-glucan content were prepared. The physicochemical properties (viscosity, peak molecular weight (M_p), and concentration (C)) of β-glucan in in-vitro-digestion extracts were evaluated, and fasting and postprandial blood glucose concentrations were measured in human subjects. Porridge and granola had the highest efficacy in attenuating the peak blood glucose response (PBGR) because of their high M_p and viscosity. β-Glucan depolymerization in bread and pasta reduced β-glucan bioactivity. Pastas, known to have low glycemic responses, showed the lowest PBGR. The analyses of these products with previously reported data indicated that 73% of the bioactivity in reducing PBGR can be explained by $M_p \times C$. Characterizing the physicochemical properties of β-glucan in bioactive foods aids functional food development.

KEYWORDS: Dietary fiber; glycemic response; nonstarch polysaccharides; nutrition; oat

INTRODUCTION

Consumption of foods rich in dietary fiber have been associated with decreased risks of developing chronic diseases such as colon cancer, atherosclerosis, diabetes, hypertension, and obesity (*1*). Particularly, many studies have shown that (1→3)(1→4) β-D-glucan, a soluble fiber present in oats and barley, is effective in attenuating postprandial glycemic and insulin responses and lowering blood cholesterol levels (*2*−*9*).

The physiological activity of β-glucan in reducing glycemic responses has been mostly attributed to its effect in increasing viscosity in the upper digestive tract (*10*−*12*). In an early study performed by Jenkins and colleagues (*13*), it was demonstrated that the capacity of certain soluble fibers, such as guar gum, to lower the postprandial glycemic response was lost after acid hydrolysis, and a relationship between viscosity and glycemic response was noted. In another study, the viscosity of β-glucan was shown to account for 79−96% of the changes in plasma glucose and insulin response to 50 g of available carbohydrates (*10*). These studies used a drink model with a fixed volume in which viscosity, concentration (C), and molecular weight (MW) can be determined. More recently, we demonstrated the principle was applicable to solid foods using oat bran muffins and correlated the glycemic response to viscosity, MW, and C of the β-glucan in an in vitro extract (*14*, *15*). In order to verify the correlation between the viscosity of the extract from the in vitro method and the glycemic responses, the procedure had to be tested with other food forms. The extractability and MW of β-glucan in different foods are likely to differ. Depolymerization of β-glucan during bread making is known to occur (*16*, *17*). The presence of other ingredients, processing methods, and storage conditions can affect either or both of these two factors and, consequently, viscosity and resultant physiological activity.

Therefore, the objectives of the present study were to analyze different types of food products with the same nutrient profile but cooked using various processing techniques (namely, granola, bread, pasta, porridge, and muffins) to evaluate the effect of food processing on the physicochemical and physiological properties of oat β-glucan and to verify whether the previously found relationship between β-glucan viscosity (or MW and C) and the blood glucose response could be also extended to other solid food products in addition to muffins. Characterizing the physicochemical properties of β-glucan in foods with high and low bioactivity will aid in the development of new functional foods and the evaluation of those currently available.

MATERIALS AND METHODS

Materials. Oatwell 22 oat bran (22% β-glucan, CreaNutrition, Zug, Switzerland) was used to make the oat products. The rest of the ingredients were locally purchased. Oat crisp bread (dried flat leavened bread) and its control wheat crisp bread were produced in a commercial bakery.

α-Amylase (EC 3.2.1.1, from human saliva), pepsin (EC 3.4.23.1, from porcine stomach), and pancreatin (EC 232.468.9, from porcine pancreas) used for the 37 °C in vitro digestion were from Sigma-Aldrich (Oakville, ON, Canada).

Experimental Foods. Product formulations are shown in **Table 1**. The source of β-glucan in oat bread and oat pasta was only Oatwell 22. Oat granola and oat porridge were made with a mix of either whole oat flakes

*Correspondent author. Tel: 519-780-8019. Fax: 519-829-2600. E-mail: toshs@agr.gc.

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Table 1. Formulations of Oat and Wheat Food Products

	oat porridge	oat granola	oat pasta	wheat pasta	wheat muffin
Oatwell 22 (g)	12	11	16		
oat bran (g)	15				
whole oat flakes (g)		28			
durum wheat flour (g)			70	80	
whole wheat flour (g)					62
corn syrup (g)	49	39			
corn starch (g)	10	7			5
dextrose (g)					11
whole egg (g)					17
egg white (g)	13	11		4	4
vegetable oil (g)	8	7	5	7	8
vanilla extract (g)	2	2			
salt (g)			1	1	1
baking powder (g)					2
water (mL)	200	74	42	27	43

(containing 4.3% β-glucan) or oat bran (containing 7.4% β-glucan), plus Oatwell (around 70% of β-glucan was derived from Oatwell in both cases). The wheat crisp bread and wheat muffin were made with whole wheat flour and both of the pastas with durum wheat flour. Other ingredients added to the oat and wheat crisp breads were rye bran, partly hydrogenated vegetable oil, emulsifier, acidity regulator, glucose−fructose syrup, yeast, salt, sugar, and water. The exact formulations of oat crisp bread and wheat crisp bread are nondisclosable proprietary information.

Oat porridge was prepared by mixing the ingredients and bringing them to a boil at medium heat setting with vigorous stirring. Then, the mix was simmered at low temperature setting with stirring for 5 min and served hot.

For the preparation of oat granola, the ingredients were mixed and baked at 350 °C for 20 min in small loaf pan molds. After cooling, the granola bar was cut into small crumbs and dried at 80 °C for 2.5 h. The granola crumbs were stored at room temperature in a sealed container.

Wheat pasta and oat pasta were prepared in a similar fashion by following the instructions of a pasta maker machine (Pastamatic MX700, Simac, Lello Appliances Co., East Rutherford, NJ). After extrusion, the pasta was dried in an 80 °C oven for 4 h and stored at room temperature for 1 to 6 weeks in a sealed container. On the day of the clinical trial, the pasta was cooked in water with 0.1% salt (NaCl) for 10 min, drained, and rinsed with hot water. A sauce of tomato paste (91.6 g) plus water (43.6 mL) was added to each serving of pasta, and the product was immediately served.

The wheat muffin was prepared fresh the day before each clinical test, according to Lan-Pidhainy et al. (*15*), and stored at room temperature in a sealed container.

The oat products were formulated to contain 4.2 g of β-glucan and 43.6 g of available carbohydrate per serving, with the exception of oat crisp bread that had 3.6 g of β-glucan and 64.4 g of available carbohydrate per serving.

The small amounts of β-glucan in wheat products came from the durum wheat or whole wheat flours. Wheat products contained similar amounts of available carbohydrates as their corresponding oat products.

Oat and wheat products were analyzed for fat (AOAC 960.39), protein (AOAC 990.03), and available carbohydrate (measured as starch + glucose [AOAC 996.11]). Dry egg white powder, vegetable oil, corn syrup, and corn starch were added in varying amounts to equalize fat, protein, and total carbohydrate content. **Table 2** shows the energy and nutrient composition for oat and wheat products.

Physicochemical Analysis of β-Glucan. Dry matter content was determined according to a standard method (AACC method 44-15A, 2000). In vitro digestion extractions of β-glucan were performed on the raw sources of β-glucan: Oatwell, oat bran, and whole oat flakes, and on the cooked oat and wheat products. β-Glucan was extracted from each batch of products using an extraction method as described by Beer et al. (*18*) except that the solids to liquids ratio was 1:5. The method involves incubating a portion of food with a series of digestive enzymes (α-amylase at pH 6.9, pepsin at pH 2, and pancreatin at pH 6.8).

The solubilized β-glucan in the supernatant from the in vitro digestion extraction was measured by flow-injection analysis (FIA, Lab Instruments, Bellevue, WA) essentially as described by Jørgensen (*19*). The total β-glucan content in the products was determined by the method of McCleary and Glennie-Holmes (*20*) using an assay kit (Megazyme International, Bray, Ireland). The glucose concentration was measured spectrophotometrically with an automated glucose oxidase procedure (*21*).

Table 2. Energy (kcal/Serving) and Nutrient Composition (g/Serving) of Oat and Wheat Food Products

	oat crisp bread	wheat crisp bread	oat porridge	oat granola	wheat muffin	oat pasta	wheat pasta
Calories	365	343	317	318	333	312	325
serving size (g)	115	107	270	97	128	366	329
fat (g)	6	4	9	9	10	8	9
carbohydrates (g)	85	85	60	62	58	71	66
fiber	22	20	17	18	12	29	22
β-glucan	4	1	4	4	0.2	4	0.4
glucose	2	1	7	6	8	3	2
starch	62	64	36	38	38	39	42
available carbohydrates[a]	64	65	43	44	46	42	44
protein (g)	15	11	16	16	15	18	18
ash (g)	3	2	2	1	2	2	3
moisture (mL)	6	4	184	9	43	267	233

[a] Calculated as starch (g) + glucose (g).

The peak molecular weight (M_p) of β-glucan was determined using high-performance size-exclusion chromatography (HPSEC) with postcolumn calcofluor addition as described by Wood et al. (*21*) but using two columns (300 × 7.5 mm) in series of Shodex OHpak KB806 M (J. M. Science Inc., Grand Island, NY) and Waters Ultrahydrogel (Waters, Milford, MA). The columns were maintained at 40 °C and eluted with 0.1 M tris buffer at 1 mL/min using a Shimadzu 10 ATVP pump. A Perkin-Elmer ISS 100 autosampler and injector were used with an injection volume of 100 μL. Postcolumn, the eluant was mixed 1:1 with 20 mg/L of calcofluor in 0.1 M tris buffer (pH 8.0) using a Waters model 590 Pump. The fluorescence was measured using an RF-10AXL fluorescence detector (excitation, 360 nm; emission, 540 nm). β-Glucan MW standards ranging from 20,000 to 1,200,000 were either provided by Megazyme, Int (Bray, Co., Wicklow, Ireland) or produced as described by Wang et al. (*22*). The MW of the standards was determined essentially as described also by Wang et al. (*22*).

Viscosity was measured by a controlled strain rheometer (TA Instrument, ARES, New Castle, DE) fitted with a cone-and-plate geometry (angle, 0.04 radians; diameter, 50 mm). Apparent viscosity was measured in a shear rate range of 400−0.1 s^{-1}. For statistical analysis, viscosities measured at the shear rate 30 s^{-1} were used primarily for consistency with earlier publications.

Clinical Trial. Twelve subjects were recruited to represent the general North American population (6 female, 6 male; aged 42.3 ± 4.2 years; BMI 28.8 ± 1.6 kg/m^2, mean ± SEM). The inclusion criteria were that subjects were males or nonpregnant females aged 18−75 years and in good health, and the exclusion/withdrawal criteria were that subjects were less than 18 years, with a known history of AIDS, hepatitis, diabetes, or a heart condition, were using medications or with any condition which might, in the opinion of Dr. Wolever, either (1) make participation dangerous to the subject or to others or (2) affect the results or subjects who cannot or will not comply with the experimental procedures, or do not follow Glycemic Index Laboratories' safety guidelines. The study was approved by the Western Institutional Review Board (WIRB PRO NUM: 971199), which meets all requirements of the US Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS), the Canadian Health Protection Branch (HPB), Canadian Institutes for Health Research (CIHR), and the European Community Guidelines. Informed written consent was obtained from all volunteers. Subjects received a financial reward for their participation.

The clinical trial was a randomized design with repeated measures. Each subject was tested on the seven different products. The wheat muffin used as a control was tested twice by each subject at the beginning and end of the study; the mean values of the two tests for each subject were used for

Table 3. Physicochemical Measurements and Postprandial Blood Glucose Response: Solubility (%), Concentration of β-Glucan in the in Vitro Extract (*C*), Peak Molecular Weight (M_p), Peak Blood Glucose Response (PBGR), and Incremental Area under the Glucose Response Curve (AUC)

	solubility (%)[a]	*C* (mg β-glucan/mL extract)[a]	$M_p \times 10^{-3}$ (g/mol)[a]	viscosity (mPa.s at 30/s)[a]	PBGR (mmol/L)[b]	AUC (mmol × min/L)[b]
oat pasta	28.8 ± 2.0 c[c]	2.0 ± 0.3 b	465 ± 9.6 b	6.50 ± 1.6 b,c	1.72 ± 0.13 b	89 ± 10 c
wheat pasta	6.70 ± 0.2 a	0.0 ± 0.0 a	33 ± 0.2 a	2.30 ± 0.1 a,b	1.93 ± 0.19 b	96 ± 12 c
oat crisp bread	61.3 ± 1.4 d	4.0 ± 0.6 d	197 ± 1.6 a	12.30 ± 0.9 d	2.90 ± 0.29 a	181 ± 21 a
wheat crisp bread	0.80 ± 0.1 a	0.0 ± 0.0 a	56 ± 0.6 a	10.10 ± 0.5 c,d	2.94 ± 0.34 a	170 ± 24 a
oat granola	34.2 ± 2.8 c	2.9 ± 0.2 c	1911 ± 17.5 c	130.6 ± 3.5 e	2.04 ± 0.21 b	107 ± 11 b,c
oat porridge	28.1 ± 5.0 c	2.3 ± 0.3 b,c	1942 ± 13.4 c	130.8 ± 0.5 e	2.22 ± 0.22 b	115 ± 11 b,c
wheat muffin	18.9 ± 1.5 b	0.1 ± 0.0 a	78 ± 0.4 a	1.80 ± 0.4 a	3.01 ± 0.30 a	144 ± 13 a,b

[a] Values are means ± SD. [b] Values are means ± SEM. [c] Values with the same letter in the same column are not significantly different ($P > 0.05$).

statistical analysis. Subjects were allowed to choose to have, with the tested product, a drink of one or 2 cups of water, coffee, or tea with 30 mL of 2% milk per cup if desired. The drink chosen remained constant for all seven tested products.

In a period of four weeks, the subjects were studied on eight separate days between 7:00 and 9:30 a.m. after 10−14 h overnight fasts. There was at least a one-day washout period between two consecutive test meals. On each test occasion, a fasting blood sample was obtained by finger-prick using a monoejector lancet device (Owen Mumford Ltd., Oxford, UK). Then the subject started to consume a test meal. At the first bite, a timer was started and additional blood samples taken at 15, 30, 45, 60, 90, and 120 min. Before and during the test, a blood glucose test record was filled out with the subject's initials, ID number, date, body weight, test meal, beverage, time of starting to eat, time it took to eat, time and composition of last meal, and any unusual activities. During the 2 h of the test, subjects remained seated quietly.

Whole blood samples were placed into 5 mL tubes containing a small amount of sodium fluoride and potassium oxalate as an anticoagulant and preservative, stored at −20 °C, and analyzed within 48 h using a glucose oxidase method (Yellow Springs Instruments, 2300 Stat). Fasting blood glucose was measured in duplicate and the average used; if the duplicates differed by >0.2 mmol/L, fasting glucose was measured in triplicate and the average of the 2 closest values used. Single postprandial glucose determinations were measured.

Statistical Analysis. Statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). The viscosities, solubilities, MW, and concentrations of β-glucan were evaluated using one-way analysis of variance (ANOVA). After demonstrating significant heterogeneity, the differences between individual means were assessed using Tukey's test to control for multiple comparisons, with the criterion for significance being 2-tailed $p < 0.05$. Means which differ by more than LSD (least significant difference) differ significantly.

Blood glucose concentration (mmol/L) was plotted against time (min). Peak blood glucose response (PBGR) and incremental areas under the glucose response curves (iAUC), subtracting area below fasting, were calculated using the method described by Wolever et al. (*23*). Blood glucose concentrations at each time and PBGR and iAUC values were compared by two-way ANOVA, examining for the effects of subject and test food, and Tukey's test as previously described.

All correlations in the figures were determined using linear regression in GraphPad Prism. Two-tailed $P < 0.05$ was considered to be statistically significant.

RESULTS

Physicochemical Data. The solubility and M_p of β-glucan in the raw sources used in this study were 38.7 ± 1.5% and 2,537,000 ± 30,000 g/mol for Oatwell, 14.7 ± 0.4% and 2,595,000 ± 51,000 g/mol for oat bran, and 14.8 ± 0.03% and 2,537,000 ± 11,000 g/mol for whole oat flakes. To facilitate the comparison of β-glucan solubilities in the cooked products, the data were calculated both as the concentration of β-glucan in the final in vitro digestion extract (mg/mL), referred to as *C*, and as the percent of the original total β-glucan solubilized during the in vitro digestion protocol (solubility). **Table 3** shows that β-glucan in oat crisp bread had the highest solubility and, therefore, the highest amount of β-glucan extracted by the in vitro digestion protocol (*C* in mg/mL). The solubilities of β-glucan in oat granola, oat porridge, and oat pasta were not significantly different ($P > 0.05$). The M_p of β-glucan in oat porridge and oat granola were the highest ($P < 0.05$) of all the products, distantly followed by the one in oat pasta. The physicochemical properties of the wheat muffin control were not significantly different from the other wheat muffins studied in previous studies ($P < 0.05$) (*14*, *15*).

Since the viscosity of random coil polymers in solution depends on concentration and molecular size, the correlation between the log ($M_p \times C$) and the log viscosity of the extract was high ($r^2 = 0.94$, $P = 0.0001$) for all oat products containing β-glucan, as expected.

Postprandial Blood Glucose Response. Mean PBGR was significantly lower ($P < 0.05$) with oat porridge, oat granola, oat pasta, and wheat pasta in comparison to that in the wheat muffin used as a control (**Table 3**). However, mean iAUC was significantly reduced only by the two types of pasta ($P < 0.05$). No significant effect was observed ($P > 0.05$) on oat crisp bread or oat pasta with the addition of β-glucan in comparison to their own wheat controls. **Figure 1** shows the changes in postprandial blood glucose with time over 2 h. The blood glucose responses after both, oat and wheat crisp bread, were significantly higher ($P < 0.05$) than that after wheat muffin at 90 and 120 min, and the responses after both, oat and wheat pasta, were significantly lower ($P < 0.05$) than that after wheat muffin at 30 and 45 min. The blood glucose increments after oat granola were less than those of wheat muffin at 30 and 45 min, and the increment after oat pasta was less than that after wheat muffin at 60 min (**Figure 1**). The glucose responses after the oat pasta and oat crisp bread did not differ significantly ($P > 0.05$) from the wheat control products for these at any point in time.

When all products were considered, with the exception of the two pastas, there was a significant inverse linear relationship between $\log_{10}$ [viscosity] and mean PBGR ($r^2 = 0.86$, $P = 0.02$), and between ($M_p \times C$) and mean PBGR ($r^2 = 0.994$, $P = 0.0002$). Pasta values seem to lie in a separate parallel curve (**Figure 2**).

A plot of the values obtained from the present study with the ones obtained previously for oat bran muffins subjected to freeze/thaw cycles (*15*) and for oat bran muffins that have been treated to vary the MW of the β-glucan (*14*) corroborates the same trends: 80% of the variation in mean PBGR in muffins, granola, porridge, and crisp bread can be explained to be due to the effect of the product of $M_p \times C$, and pastas seem to behave in a comparable pattern starting at a lower value of PBGR (**Figure 3A**).

Differences in the β-glucan efficacy in reducing glycemic response could be standardized when food products are referred to their own proper controls. Thus, a significant correlation ($r^2 = 0.73$, $P < 0.0001$) between the physicochemical properties of β-glucan and its bioactivity is observed

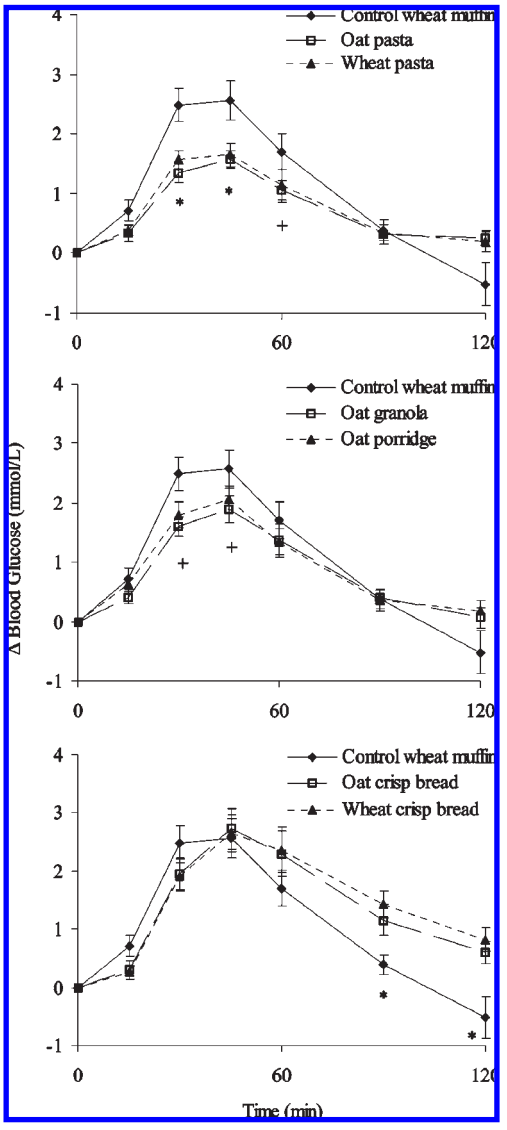

Figure 1. Blood glucose increments after tested foods and wheat muffin used as a control. Values are the mean ± SEM for $n = 12$ subjects. *, both tested foods are significantly different from wheat muffin ($P < 0.05$). +, oat granola or oat pasta is significantly different from wheat muffin ($P < 0.05$).

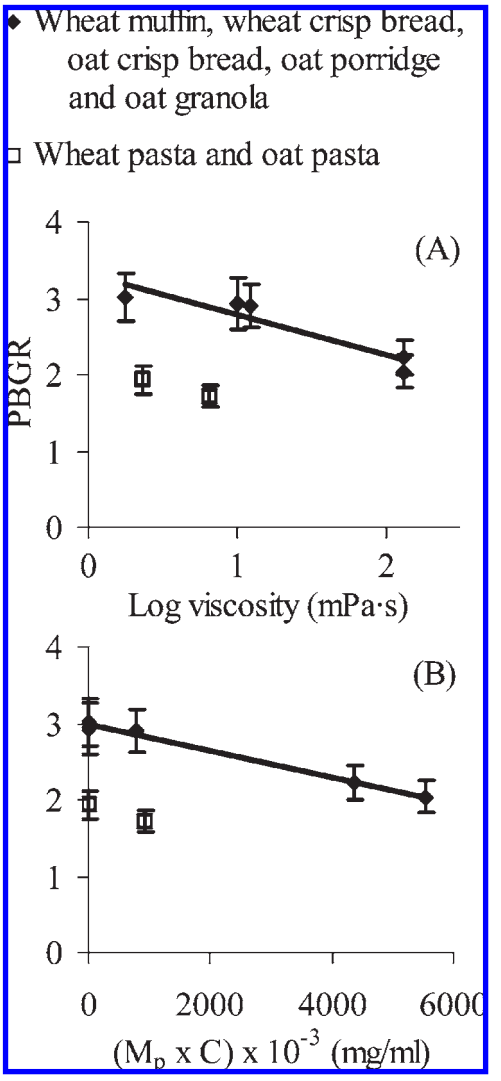

Figure 2. Correlations between (**A**) log viscosity (mPa·s) at 30 s^{-1} and mean peak blood glucose rise (PBGR) ($r^2 = 0.86$, $P = 0.02$) and (**B**) the product of peak molecular weight times concentration ($M_p \times C$) and PBGR ($r^2 = 0.994$, $P = 0.0002$) for muffins, crisp bread, granola, and porridge. Pastas are not considered in the linear regressions. Values are means ± SD.

for all food products (**Figure 3B**) when the wheat muffin control is used as a reference for oat granola, oat porridge, and oat muffins, and oat pasta and oat crisp bread are compared to their own wheat controls.

DISCUSSION

The three sources of β-glucan used: Oatwell, oat bran, and whole oat flakes, had a mean M_p ± SD value of 2,556,000 ± 33,000 g/mol, which falls within the reported range of 2,000,000−3,000,000 g/mol (*21*, *24*). The MW of β-glucan in processed oat foods is usually smaller than the MW in unprocessed whole oats. The physical state of the β-glucan in the raw material, β-glucanase activity, processing, and storage conditions affect the solubility and MW of β-glucan (*18*, *25*−*27*). In this research, the M_p values of oat porridge and oat granola (**Table 3**) were greater than 1,900,000 g/mol, which indicates that little depolymerization occurred during the food processing of these two food products, as previously reported for oat porridge by Åman et al. (*25*). Conversely, for oat crisp bread and oat pasta, the β-glucan reductions in M_p were 92 and 82%, respectively, from the original oat sources. These values are similar to those previously reported in oat/wheat and barley/wheat breads (*16*, *17*), and pastas (*25*). The low MW of β-glucan in oat bread and oat pasta has been attributed to the presence of β-glucanases in the wheat flour used to make oat bran bread (*17*, *25*−*28*). Anderson et al. found that the MW in crisp breads decreased with increasing mixing and fermentation time of the dough (*26*).

In this research, β-glucan was also subjected to two other very different heat treatments: in oat granola, the batter was baked at 177 °C per 20 min in dry heat, and in oat porridge, the ingredients were boiled at 100 °C and then simmered for 5 min in wet heat. Both had short preparation times which resulted in similar high β-glucan M_p values. It would seem that the glycosidic linkages of β-glucan are relatively stable during either of these heat treatments, whereas prolonged treatment at lower temperatures, in the presence of wheat flour, such as in the proofing process in bread or during the kneading and extrusion processes in pasta at which enzymes remain active, may result in extensive degradation (*25*).

As observed in **Table 3**, the solubility of β-glucan in oat crisp bread was the highest of all foods. Trogh et al. reported an

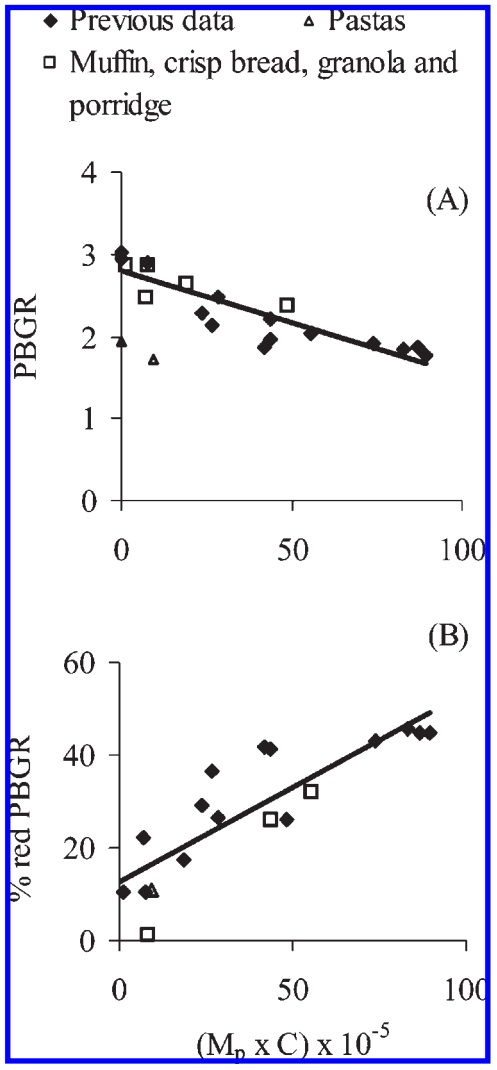

Figure 3. Correlations between the product of peak molecular weight times concentration ($M_p \times C$) and (**A**) the mean peak blood glucose response (PBGR) ($r^2 = 0.80$, $P < 0.0001$) and (**B**) the percent in reduction of the PBGR ($r^2 = 0.73$, $P < 0.0001$) for muffins with different M_p from previous data (*14*, *15*) and muffin, crisp bread, granola, porridge, and pasta from the current study.

increase of water extractability of β-glucan during the mixing of dough (*17*). During processing, the action of enzyme causes depolymerization and releases polysaccharides from the cell wall structure, thereby increasing their solubility. In the case of oat pasta, depolymerization of β-glucan also occurred but without concurrent increase in the solubility of the polymer. Relationships between MW and solubility are not straightforward. Although β-glucanase may initially help release β-glucan from the cell wall, increasing solubility, as degradation proceeds solubility may decline because of the formation of insoluble β-glucan aggregates (*14*).

In addition, previous studies have reported that the oat source could also be a determinant factor in the solubility of β-glucan. For example, the milling of oat groat can improve the extractability of β-glucan, by reducing the particle size (*2*). In this study, the high β-glucan oat bran (Oatwell) used was previously milled and had a higher β-glucan solubility (39%) than the coarser oat bran and whole oat flakes (15% for both). However, neither the raw source of oats (whole oat flakes and Oatwell mix in oat granola or the differently milled oat bran in oat porridge or oat pasta) (**Table 1**) nor the moisture content of the product during cooking (low for oat granola vs high for oat porridge) made a significant difference in the extractability of β-glucan in these three cooked products.

In the current study, a significant relationship ($r^2 = 0.94$, $P = 0.0001$; equation: $\eta = 1.5 \log (M_p \times C) - 7.9$) is maintained between the logarithm of the product of β-glucan concentration (C) and M_p, and the logarithm of the extract viscosity (η). Although the high value in the correlation should be carefully considered because there was no data at medium log ($M_p \times C$) or log viscosity, this relationship in random-coil polymers is well known, and it has been previously published (*29*). The fact that there are no intermediate values of M_p in these products confirms the high β-glucanase activity coming from the wheat flour in pasta and crisp bread, which poses a challenge in making bioactive oat crisp bread or bioactive oat pasta.

With regard to the clinical trial, our results confirm previously reported data that show that pastas are lower glycemic response foods than other foods with equivalent content of available carbohydrates (*30*−*32*). The low glycemic response of pasta has been linked to a reduced and relatively stable progressive liberation of sugars during digestion. This may be attributed, in part, to the compact structure of pasta resulting from the extrusion process, which brings about a dense protein network entrapping starch granules and thereby delaying amylolysis (*33*). Studies have illustrated that the in vitro sugar release and/or glycemic response of pasta can be lowered further, by the addition of soluble fiber including guar gum (*34*, *35*), inulin (*36*), and β-glucan (*6*, *37*). Cleary et al. found that the addition of barley β-glucan (3.7 to 6.4%) to pasta generally attenuated reducing sugar release during in vitro digestion, although not consistently until after 150 min of digestion. In this study, the magnitude of the reduction was simply related to the amount of barley β-glucan added (*37*). Investigation of pasta microstructure and characterization of starch gelatinization events indicated that a combination of changes to the starch-protein matrix and the high water binding capacity of β-glucan altered the physicochemical properties and digestibility of the pastas (*37*). In our research, there was a reduction of 11% in the PBGR of the pasta with the addition of β-glucan but this reduction was not large enough to be significant in a single comparison ($P > 0.05$, $n = 12$). Neither Bourdon et al. (1999) nor Holm et al. (1992) could find significant differences in the glucose responses of pastas enriched with β-glucan and their wheat pasta controls (*38*, *39*). The low activity of β-glucan in oat pasta is most probably due to its partial depolymerization by the enzyme activity initially present in the durum wheat flour. Since the previous studies did not measure MW or solubility, these studies can not be compared directly with ours (*6*, *37*, *38*). We would like to emphasize the importance of measuring the MW and extractability and/or viscosity of β-glucan in the products in support of determinations of physiological activity, otherwise sources of variability can only be speculated on.

In the case of the crisp breads, the blood glucose responses after both, oat and wheat, were significantly higher ($P < 0.05$) than after wheat muffin at 90 and 120 min (**Figure 1**) most probably because the amounts of available carbohydrates of the two crisp breads were higher than in wheat muffins (**Table 2**). The evident lack of bioactivity of β-glucan in the oat crisp bread, relative to its own wheat control, is clearly a result of the previously mentioned extensive depolymerization of the β-glucan brought about by the enzymes of the whole wheat flour (*17*, *25*−*28*). This resulted in a final β-glucan MW of 197,000 g/mol in the test crisp bread (**Table 3**), which reduced the bioactivity despite having the highest solubility of all food products. Frank et al. (*28*) and Cleary et al. (*40*) found no significant differences between the physiological effects of two types of bread with different calcofluor

average MW; however, the first study measured a chronic effect in the reduction of blood glucose concentrations rather than acute glycemic responses. Some other studies have found a reduction in the postprandial blood glucose response in breads and other food products containing wheat flour enriched with oat or barley β-glucans (*5*, *41*, *42*), but the MW of β-glucan was not measured in these studies.

Thus, dose, MW, and extractability of β-glucan are important parameters in defining the physiological activity of this polysaccharide (*43*). In the current study, the oat crisp bread contained 3.6 g of β-glucan, and the available carbohydrates were almost 50% higher than those in the other products. The high content of available carbohydrates and low MW of the β-glucan probably prevented any detectable lowering of glycemic response from viscosity.

Although traditional oatmeal porridge is a moderately low glycemic index food (*44*), porridge meals made from oat bran or from wheat flour (cream of wheat) to which β-glucan isolate was added had a low glycemic response relative to that of cream of wheat and bread (*45*). The size of the effect is influenced by β-glucan content (*46*) and particle size; intact cereal grains have lower glycemic responses than their milled products (*47*). Aston et al. reported that the glycemic index of the porridge meal made with the intact jumbo oats was significantly lower than that made with the smaller more finely processed oats (*31*). In our study, oat porridge (where 29% of the total β-glucan came from coarse oat bran) and oat granola (where 33% of the total β-glucan came from whole oat flakes) showed similar PBGR and, both of them, were significantly lower than the PBGR of the reference wheat muffin control. To our knowledge, no study on the reduction of glycemic responses by β-glucan enriched granolas has been reported before, although a similar product, a β-glucan bar, made of cooked−extruded oat bran concentrate, wheat flakes, and fructose, elicited a significant reduction in the glycemic response in comparison to that by white bread in type 2 diabetic subjects (*7*).

One of the main objectives of this study was to verify that the correlation, previously observed in oat bran muffins subjected to freeze/thaw cycles (*15*) and in oat bran muffins that have been treated to vary the MW of the β-glucan (*14*), between the extract viscosity (or $M_p \times C$) of the in vitro digestion extract and the glycemic response could be extended to other solid food products. Linear regression analysis demonstrated that oat granola, oat porridge, and oat crisp bread behave the same as oat muffins; as the product of the solubilized β-glucan times M_p increased, the extract viscosity increased ($r^2 = 0.86$, $P = 0.02$), and the PBGR diminished ($r^2 = 0.994$, $P = 0.0002$) (**Figure 2**). There was a trend in pastas to act similarly to the other products but in parallel fashion (**Figure 2**), which is not surprising considering the lower glycemic response often reported for this product even without β-glucan (spaghetti made with durum wheat and boiled 10−15 min: 64 ± 5), in comparison to higher values for bran muffin (85 ± 8), porridge made from rolled oats (83 ± 5), and wheat crisp bread (79) (*48*). This is an indication that when estimating β-glucan bioactivity, other important factors should be considered such as the effect of microstructure and/or starch digestibility as previously mentioned.

Examination of the data from our three studies, Lan-Pidhainy et al. (*15*), Tosh et al. (*14*), and the present study, corroborates the relationship between $M_p \times C$ and the PBGR for muffins, granola, porridge, and bread, which follows the equation: PBGR = −0.01 ($M_p \times C$) + 2.8 (**Figure 3A**). According to **Figure 3A**, we can conclude that the starting point of β-glucan bioactivity (ordinate in the equation) is given by the nature of the food itself (probably linked to the original glycemic value of the control product), which is dependent on microstructure and/or starch digestibility, and the slope in the reduction of glycemic response is proportional to the viscosity of β-glucan, which can be expressed as MW times concentration. The latter two intrinsic physicochemical properties of β-glucan are mainly influenced by the raw materials used and their processing history.

Such intrinsic differences arising from microstructure and/or starch digestibility inherent to the food products themselves could be standardized when the β-glucan activity is measured as a percent of reduction of the PBGR in comparison to the appropriate equivalent food controls. The fact that oat granola and oat porridge fit in the equation with the oat muffins suggests that the functionality of β-glucan in these products is mainly dictated by the same equation which is a function of $M_p \times C$, and the differences in structure/starch digestibility (glycemic values) between their wheat control products are not as important as in the case of pasta. Therefore, in the calculation of percent of reduction of PBGR, the wheat muffin control value was used as reference for oat granola and oat porridge, and oat pasta and oat bread were referred to their own wheat controls. With this approach, the combined data thus suggests that 73% of the variation in reducing the PBGR can be explained by the product of $M_p \times C$ (**Figure 3B**; equation, PBGR = 0.41($M_p \times C$) + 12.6).

In summary, this study has been successful in evaluating the physicochemical changes of β-glucan after different processing methods and in verifying the existence of a relationship between these physicochemical characteristics and the β-glucan functionality in reducing the glucose response rise in five different food products: granola, porridge, muffins, crisp bread, and pasta.

To our knowledge, no previous study has been done in such a complete manner: evaluating the physicochemical characteristics of β-glucan (MW, viscosity and solubility) and the glucose response measured in a clinical trial with humans in as many different food products containing β-glucan. Pieces of information are reported elsewhere but not a complete set of data that facilitates the comparison among all these different variables and permits the establishment of significant correlations between the chemistry of β-glucan and its bioactivity. The relevance of finding an equation that could estimate the bioactivity of β-glucan is crucial for the development of new functional fiber products and the evaluation of the currently available food products.

Additional research is currently ongoing on the evaluation of the differences on starch gelatinization, starch digestibility, and the microstructure of these foods to be able to have a better understanding of β-glucan functionality.

ABBREVIATIONS USED

C, concentration; MW, molecular weight; M_p, peak molecular weight; PBGR, peak blood glucose response.

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