

**Title:** At a high dose even partially degraded beta-glucan with decreased solubility significantly reduced the glycaemic response to bread.

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## **Abstract**

Cereal beta-glucan has been shown to reduce post-prandial glycaemic responses, which makes it an interesting ingredient to improve the health impact of bread, a staple food with a high glycaemic index (GI). Here we compare the ability of different wheat-based breads prepared with oatbran concentrate and barley flour and a Norwegian type of soft wrap (lompe) for their ability to reduce glycaemic responses in healthy adults. Both breads with the highest beta-glucan content (3.8 g per serving) significantly reduced peak blood glucose rise (PBGR), incremental area under the blood glucose curve (iAUC) and GI compared to wheat control regardless of beta-glucan  $M_w$  and solubility. At a medium dose of 1.7 g per serving breads with beta-glucan of high MW and solubility significantly lowered iAUC, but not GI or PBGR compared to white bread. In contrast to previous studies, no significant correlation between viscosity after *in vitro* digestion and any of the glucose variables was found. However, the amount of soluble beta-glucan per serving was inversely correlated with GI. Lompe had a similar medium GI (63) than the high dose beta-glucan breads (56 and 64). However, while “lompe” had significantly lower amounts of rapidly digestible starch, no differences in starch digestion were found between the different breads. Instead, increased local viscosity at the intestinal border (e.g. soluble beta-glucan interacting with the mucus layer), dilution of nutrients (higher water content and serving size) and/or reduced gastric emptying may explain the lower glycaemic responses of high dose beta-glucan breads.

## **1. Introduction**

Glycaemic response (GR) is the post-prandial rise in blood glucose after ingestion of a food or meal containing available carbohydrate. The extent of the GR to a food does not only depend on the amount of available carbohydrate, but also on its physiological properties<sup>1, 2</sup>. The glycaemic index (GI) is used to compare the carbohydrate quality of different foods. The GI compares the incremental area under the blood glucose response curve (iAUC) elicited by a test food with the iAUC elicited by a reference food (glucose or white bread) containing the same amount of available carbohydrate (usually 50 g) in the same subjects<sup>3</sup>. At least 10 subjects are needed to get good estimates of the GI of a test product (average of all subjects)<sup>4, 5</sup>. Several health benefits including reduced risk of cardiovascular disease, metabolic syndrome and type II diabetes have been associated with low-GI diets<sup>3, 6-8</sup>. However, there is a lack of low GI foods and many staple foods such as bread have a high GI<sup>9</sup>. Nevertheless, there is a huge potential for low GI staple foods such as bread since exchanging common bread with low GI bread made with whole cereal kernels for only three weeks was enough to improve insulin sensitivity in patients with impaired glucose tolerance<sup>10</sup>.

Even though wholegrain wheat breads contain relatively high amounts of dietary fiber (usually mostly insoluble), the GI of wholegrain wheat bread is similar to that of white wheat bread<sup>11, 12</sup>. Intact cereal kernels are effective in reducing glycaemia, presumably because the intact botanical structure reduces starch accessibility<sup>13</sup>. Intact kernels and organic acids have been used to create low GI breads<sup>11</sup>. However, not all consumers like breads with intact kernels or an acidic taste. Instead, soluble dietary fibers such as cereal beta-glucan can be used to produce low GI breads<sup>11</sup>. Many of the proposed mechanisms by which soluble dietary fibers may influence glycaemic response are related to their viscosifying properties and include delayed gastric emptying, changes in hormonal regulation, delayed or reduced starch degradation and delayed sugar absorption<sup>14</sup>.

Among different soluble dietary fibers, cereal beta-glucans are especially interesting, due to their high natural content (4-7 %) in the cereals barley and oat <sup>15</sup> and their health claims approved by the European Food Safety Authority (EFSA) on reduction of blood cholesterol levels <sup>16,17</sup> and reduction of post-prandial glycaemic responses <sup>18</sup>. However, the use of the EFSA claim “reduces post-prandial glycaemic response” requires foods to contain 4 g beta-glucan per 30 g available carbohydrate. This is difficult to achieve in bread, but improvements in dry fractionation of cereal grains have resulted in an increased availability of high beta-glucan (with 10 to 30% beta-glucan content) flours from barley and oat, which may facilitate the production of foods fulfilling the criteria of EFSA health claims.

However, clinical studies have shown that beta-glucan molecular weight (MW), solubility and viscosity (after *in vitro* digestion) are important parameters influencing the glycaemic response to e.g. muffins, extruded breakfast cereals and granola with equal beta-glucan contents <sup>19-22</sup>. To ensure optimal reduction of GR or facilitate similar effects at lower doses, beta-glucan MW and solubility in food products must be optimized. During bread production, beta-glucans are degraded by endogenous enzymes, but strategies that minimize this reduction have been developed and employed for barley bread <sup>23</sup>.

In this study, we have used barely flour and oatbran concentrate to produce breads with different contents of beta-glucan and varying physicochemical properties. The breads were tested *in vivo* alongside a Norwegian type of unleavened potato-cereal flour, tortilla-like, soft wrap known in Norway as ‘lompe’ for their ability to reduce GR in normal healthy adult humans. Further the GI of lompe has never before been measured. *In vitro* digestion protocols were used to investigate the effect of beta-glucan on viscosity during digestion and on starch digestibility. We have used this information to discuss and give a glimpse into the potential mechanisms by which beta-glucans in bread may elicit their hypoglycaemic effect.

## **2. Material and Methods**

### **Experimental foods**

Commercial wheat flour of high protein strength (Lantmännen Cerealia, Oslo, Norway) was used to produce the experimental breads. Wheat flour was used alone to produce a white bread control. To produce a barley bread, 40% of the wheat flour was substituted with a barley flour produced on a laboratory hammer mill (Retsch, Model ZM100, Retsch GmbH, Haan, Germany) with a 0.5 mm mesh from barley flakes prepared from de-hulled Olive (a Norwegian barley variety) micronized and flaked by Lantmännen Cerealia (Moss, Norway). The barley bread was designed to fulfill the criteria for the EFSA health claim on reduction of LDL-cholesterol for beta-glucan and contained 1g beta-glucan per serving <sup>17</sup>. An oatbran concentrate (OBC) containing 14 g beta-glucan per 100 g (SweOat bran BG14 bakery, Swedish oat fiber, Bua, Sweden) was used to prepare two breads that fulfill the criteria for the EFSA health claim for beta-glucan on reduction of postprandial blood glucose rise <sup>18</sup> and one bread with a lower beta-glucan dose. To achieve the 4 g beta-glucan per 30 g available carbohydrate required for the “glucose” health claim, 50 g OBC and 50 g wheat per 100 g flour were used. The OBC bread with a lower beta-glucan dose (lowOBCB) was prepared with 25 g OBC and 75 g wheat per 100 g flour.

All breads contained 1 g dry yeast (Idun, Oslo, Norway), 1.5 g NaCl and 1 g fat (vegetable fat and oil, A/S Pals, Oslo, Norway) per 100 g flour. The barley bread was produced by a previously optimized baking procedure, which minimizes beta-glucan molecular weight reduction <sup>23</sup>. The procedure involves the development of a pure wheat flour dough, which is then fermented for 1h before the barley flour and additional water is added. The same approach was used to prepare one of the high dose OBC breads (optimalOBCB) and the lowOBCB. All wheat flour doughs were prepared with 58.4% water (on flour basis) in a spiral mixer (Diosna sp12, Diosna, Osnabrück, Germany) for 2 min at low and 6 min at high speed. Dough temperature after mixing

was  $27 \pm 1$  °C. The wheat flour doughs were fermented for 1h at 27°C and 70% RH in a fermentation cabinet (Lillnord AS, Odder, Denmark). The OBC was pre-hydrated for 1h at RT with 200 g water per 100 g OBC. The water addition to OBC was optimized empirically to achieve acceptable dough handling properties and bread quality. The pre-hydrated OBC was then incorporated into the fermented wheat flour dough for 2min at low speed (Diosna sp12). One of the breads with high content of OBC was prepared by mixing all ingredients together (degradedOBCB). Doughs were divided into pieces of 243 g (optimalOBCB and degraded OBCB), 162.8 g (lowOBCB), 128.6 g (barley bread) and 110 g (white bread), corresponding to 50g available carbohydrate, molded, placed in small steel pans and proved at 30°C and 70%RH for 30 min (barley bread), 45 min (white bread, lowOBCB, optimalOBCB) or 5h (degradedOBCB). Breads were baked in an rotating hearth oven (Revent type 626 G EL IAC, Revent international, Väsby, Sweden) for 20 min. Immediately after the loaves were put into the oven, the temperature was reduced from 240 to 220 °C and steam (0.5 L water) was injected during the first 10 sec. One hour after baking weight (scale) and volume (TexVol BVM –L 370, TexVol instruments AB, Viken, Sweden) of the breads was determined.

In addition to the five different breads, commercial lompe made from potato (pre-cooked and cooled) and spelt flour (a brief description of the baking process is given in <sup>24</sup>) was obtained from Buer (Speltlompe, Buer AS, Askim, Norway) and included in the clinical study. The lack of reliable GI data for common Nordic food items has long been recognized <sup>25</sup>. The GI of “lompe” has (to our knowledge) never been determined, despite the fact that the high content of cooked and cooled potatoes makes “lompe” a very interesting product for glycaemic response measurement.

All six test products (five breads and one “lompe” were frozen in a rapid freezer (Blast freezer, Lillnord, Odder, Denmark) stored and shipped frozen prior to consumption in the clinical trial and analysis.

## **Chemical composition**

The moisture content of the test foods and the chewed expectorated test samples was determined by a two-step gravimetric method (AACC 44-15A). For all other chemical analyses, test foods were freeze dried and milled on a laboratory mill (Retsch, Model ZM100). The nutrient composition per fresh weight was calculated by using the water content of the test foods and the residual moisture in the freeze dried samples, which was determined using an IR-dryer (Satorius Moisture Analyser YTC01L, Satorius AG, Goettingen, Germany) prior to each chemical analysis. Protein was determined by the Kjeldahl method using a factor of N x 6.25. Fat was determined gravimetrically following acid hydrolysis, extraction into diethyl ether and petroleum ether and evaporation. Total dietary fiber was determined gravimetrically by AOAC 985.28 and total beta-glucan was determined by the enzymatic method (AOAC 995.16) based on a cereal mixed-linkage  $\beta$ -glucan kit from Megazyme (Megazyme International, Bray, Ireland). Available and resistant starch were determined according to AOAC 2002.02 using a resistant starch assay kit from Megazyme. Ash (total mineral content) was determined as residue after heating to 550°C. Total energy content per serving was calculated from the nutrient composition according to EU Council Directive 1169/2011 and available carbohydrate was calculated as described by Brouns et al. <sup>4</sup>.

## **Physicochemical analysis of beta-glucan in breads**

To determine the physicochemical characteristics of beta-glucan in the breads under physiological conditions, all test foods were subjected to an *in vitro* digestion procedure as described earlier <sup>26</sup>. After digestion, samples were centrifuged and the rheological properties of the supernatants were characterized using a Physica MCR 301 rheometer fitted with a double gap (DG26.7) geometry as previously described <sup>26</sup>. Beta-glucan concentration and weight average molecular weight ( $M_w$ ) were determined in the extracts after digestion using SEC with

post column calcofluor detection as described earlier <sup>26</sup>. Beta-glucan concentrations in the extracts were used to calculate beta-glucan solubility under physiological conditions.

### ***In vitro* starch digestibility**

As a potential *in vitro* predictor of glycemic response the amount of rapidly digestible starch (RDS) and the kinetics of glucose release from the test products were determined <sup>27-29</sup>. The *in vitro* digestion protocol employed for this purpose was based on the method by Monro et al.<sup>29</sup> with modifications. The different breads and lompe were thawed overnight, chewed until the urge to swallow and then expectorated. The expectorated material was thoroughly mixed and 2 g aliquots were weighed into 50mL centrifuge tubes in duplicates for each time point. The samples were first subjected to a simulated gastric digestion at pH 3 and 37°C for 1h. Buffer and enzyme (pepsin) addition were as earlier described <sup>26</sup> and according to the Infogest protocol <sup>30</sup>. After the gastric phase, 4mL pre-warmed (37°C) 0.1M Na-maleate buffer pH 6 with 0.2% Na azide and 1mM CaCl<sub>2</sub> containing 200 U/mL pancreatin (based on trypsin activity, P1750 from porcine pancreas, Sigma-Aldrich, St Louis, US) were added to each tube, together with 50µL amyloglucosidase (3300 U/mL on soluble starch, Megazyme) and pH was adjusted to 6 by adding pre-determined amounts of 1M NaOH. Tubes were vortex mixed and placed horizontally in a shaking incubator (Innova 40, Incubator Shaker Series, New Brunswick Scientific, Edison, New jersey, US) at 175rpm and 37°C. After 120 min incubation, the two remaining tubes were vortex mixed vigorously before incubating further for a total of 180 min. The reaction was stopped after 0, 10, 20, 40, 60, 120 and 180 min by adding 32 mL ethanol to each of the two tubes per time point. Tubes were centrifuged and the supernatants were diluted with water (1:10) before aliquots of 100µL were mixed with 500µL 200mM Na acetate buffer pH 5.2 containing 33 U/mL amyloglucosidase (Megazyme). After 20 min incubation at 50°C, released glucose was measured spectrophotometrically using a glucose oxidase assay (Megazyme).



## **Clinical trial**

The clinical trial was based on international recommendations for glycaemic index testing <sup>4</sup>. Fifteen healthy subjects were recruited and fourteen (12 females, 2 males) completed the study. The inclusion criteria were age (18-65 years), BMI (18-27 kg/m<sup>2</sup>), gender (both male or female) and self-diagnosis as healthy (medical questionnaire). Subjects with a history of diabetes or subjects that had consumed anything apart from water 12h prior to the test, were excluded from the study. Informed written consent was obtained from all volunteers before study start. All clinical testing was conducted at Leatherhead Research Ltd, UK within a three month period between October-December 2015.

The mean age of the subjects was 44.76 years (SEM 3.69) with a mean BMI of 24.26 (SEM 0.44) kg/m<sup>2</sup>. One subject did not consume one of the breads, another subject tested the reference glucose only twice rather than three times. Otherwise, all 14 subjects completed all nine visits. The study was a randomized block design with repeated measures with each subject testing the six different breads once and the glucose control three times (in the beginning, middle and end of the study). Mean values of the three glucose reference tests of each subject were used for statistical analysis.

Tests were conducted in the morning after an at least 12h overnight fast. Subjects were instructed to avoid strenuous exercise, smoking and alcohol consumption the evening before a test and consume a similar carbohydrate-based evening meal before each test session. There was at least a 48h wash out period between the tests. Subjects had to consume the test products within 15 minutes with 250 mL of water. Since it was impossible for some subjects to consume the initial portion size (corresponding to 50 g available carbohydrate) of the high beta-glucan breads within 15 minutes, the portion size was decreased to contain 25 g available carbohydrate for all breads and the glucose reference. Finger prick capillary blood samples were taken at 0, 15, 30, 45, 60, 90, 120 and 180 min. Blood samples were collected into small tubes containing

lithium-heparin and centrifuged at 3000 rpm for 10 min to separate plasma. The plasma samples were then analysed for glucose by an YSI 2300 Stat Plus Glucose and Lactate Analyser (sensitivity 0-50 mmol/L and margin of error +/- 2%).

### **Calculations and statistical analysis**

The incremental area under the glucose response curve (iAUC) above baseline was calculated from 0-120 min using the standard trapezoid geometric method as previously described<sup>31</sup>. Peak blood glucose rise (PBGR) was calculated as the differences of each subject's peak and fasting glucose values. The GI was calculated by expressing the iAUC for the test food in each subject as a percentage of the same subjects mean reference (glucose) iAUC. The GI of the food was the mean of the GI values calculated for each subject. The mean and coefficient of variation (CV = 100xSD/mean) of within-individual iAUC values for repeated measures of the reference food (25g glucose) was calculated for each subject. The mean CV for the subject group was with 21.1 below the upper recommended threshold of 30<sup>5</sup>. Individual values of iAUC or GI greater than the mean plus 2 times standard deviation (SD) were considered outliers and removed from the final results as previously recommended<sup>5</sup>. The influence of this outlier removal is discussed in the results section.

All statistical analysis were performed using Minitab version 18. Statistical differences between mean iAUC, GI and peak blood glucose rise (PBGR) for each test food were assessed by repeated measures ANOVA using a general linear model with test food (fixed) and subject (random) as factors. Comparisons between test foods were made with the *post hoc* Tukey pairwise comparison test at a confidence interval of 95%. For the five different breads (not lompe), linear regression analysis and Pearson correlation were used to examine the relationship between the glucose variables (iAUC, GI and PBGR) and different bread characteristics (beta-glucan: Mw, concentration after *in vitro* digestion (c), viscosity after *in vitro* digestion, total amount, and amount of soluble beta-glucan, Mw x c and Mw x amount of soluble beta-glucan

in linear and log<sub>10</sub> scale and serving size). A value of  $p < 0.05$  was considered to be statistically significant.

### **3. Results and Discussion**

#### **Postprandial blood glucose response**

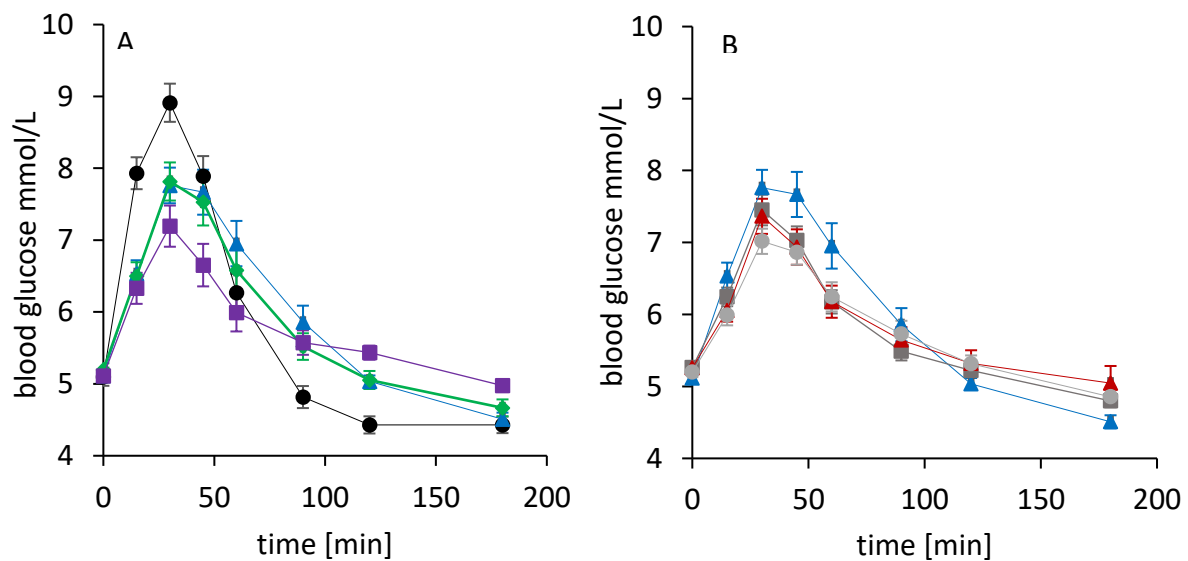
The blood glucose rise after ingestion of the test foods differed substantially from the blood glucose rise of the glucose reference (Figure 1 and Table 1). All test foods elicited a significantly lower peak blood glucose rise (PBGR) than the glucose reference (Table 1). For barley bread and lompe, outlier removal changed the PBGR from 2.82 to 2.68 (outlier 4.86) and from 2.19 to 2.04 (outlier 4.34), respectively. Among the test foods, white bread had the highest PBGR, followed by barley bread and lowOBCB. The PBGR elicited by optimalOBCB, degradedOBCB and lompe was significantly lower than for white bread (Table 1), although there was no significant difference in PBGR between these 3 types of bread (Table 1)

For lowOBCB, degradedOBCB and optimalOBCB, outlier removal changed the average iAUCs from 126.7 to 104.5 (outliers 270 and 272), 114 to 107.1 (outlier 211) and 117.3 to 106.8 (outlier 243), respectively. The iAUCs elicited by the different test foods were lower than for the glucose control (Table 1). However, for white bread and barley bread, this difference was not statistically significant. Compared to white bread, all test foods, except barley bread, resulted in a significantly lower iAUC (Table 1). For lowOBCB this difference was only statistically significant after the removal of outliers.

For white bread, lowOBCB, optimalOBCB and barley bread, outlier removal changed the GI estimates from 94.3 to 84.1 (outlier 237), 68.6 to 64.9 (outlier 121), 60.9 to 56.8 (outlier 109) and 77.1 to 71.8 (outlier 150), respectively. However, the removal of outliers did not change the differences between GI values significantly (Table 1). The GI value for white bread of 84.1 was relatively high compared to mean GI values for white bread (72.5 and 75) obtained by an

inter-laboratory study or published in the international table of GI and GL<sup>32, 33</sup>. However, the published mean GI values (72.5 and 75) are for shop bought white bread. Industrially produced white wheat bread normally contains different additives, such as the emulsifier diacetyl tartaric acid esters of monoglycerides (DATEM). DATEM slows down staling by interfering with starch retrogradation<sup>34</sup> and has been shown to reduce the GI of white bread<sup>11, 34</sup>. In comparison, a GI of 95 was reported for French baguette produced without additives<sup>12</sup>. Furthermore, specific loaf volume influences the GI of white bread<sup>35</sup>, which further complicates the direct comparison of GI values. Nevertheless, a white bread produced without additives and with a similar specific volume (3.17 mL/g) than the white bread in our study (3.6 mL/g) also showed a similar GI of 86<sup>35</sup>.

The barley bread had a GI of 72, which was lower than for white bread with a GI of 84. However, the difference was not statistically significant, and the GI of the barley bread was still in the range of high GI foods (> 70). All three breads with OBC and lompe had GI in the medium range (55-70). However, the difference in GI for lowOBCB and white bread was not statistically significant.



**Figure 1:** Changes in blood glucose with time within 2h postprandial. Values are mean +/- SEM. A: glucose reference (black dots), white bread (blue triangles), barley bread (green

diamonds), lompe (purple squares). B: white bread (blue triangles), lowOBCB (dark grey squares), degradedOBCB (red triangles), optimalOBCB (light grey dots).

**Table 1:** Postprandial blood glucose response<sup>a</sup>

Food	PBGR (mmol/L) <sup>b</sup>	iAUC (mmol x min/L)	GI (% of control)
Control (glucose)	3.94 ± 0.24 a	180.4 ± 14.9 a	100
White bread	2.93 ± 0.27 b	165.8 ± 20.4 a	84 ± 7 a
Barley bread	2.68 ± 0.26 bc	139.9 ± 18.0 ab	72 ± 6 ab
lowOBCB	2.51 ± 0.25 bcd	104.4 ± 9.3 b	65 ± 4 ab
degradedOBCB	2.28 ± 0.21 cd	107.1 ± 10.3 b	64 ± 5 b
optimalOBCB	2.11 ± 0.19 d	106.8 ± 13.9 b	57 ± 4 b
Lompe	2.04 ± 0.27 d	113.4 ± 16.4 b	63 ± 6 b

<sup>a</sup> values are mean values ± SEM for all subjects after outlier correction (n= 14-12 ; values higher than mean + 2 times SD were removed). Values not followed by the same letters in columns were significantly different at p < 0.05.

<sup>b</sup> Peak blood glucose rise: difference between peak and fasting blood glucose

### Effect of protein and fat

Since fat and protein can influence the GR to a test product<sup>3,36</sup>, the macronutrient composition of different test products is often standardized for example by adding egg white powder to equalize the protein content<sup>22</sup>. In the present study, we kept the ingredients as simple as possible and exchanged wheat flour for barley flour or OBC without any further adjustment of macronutrient composition. Due to the higher content of fat and protein in OBC compared to wheat flour, the breads prepared with OBC contained slightly higher amounts of protein (up to 4.2 g difference per serving) and fat (up to 2.9 g difference per serving) compared to the white bread control (Table 2). The effect on GR of adding fat and protein to glucose has been estimated<sup>36,37</sup> and used successfully to calculate the GI of meals containing different amounts of protein and fat<sup>3,31</sup>. We calculated the potential reduction of 4.2 g protein and 2.9 g fat on the GI of the white bread control by using the mean adjustment factors of 0.29%/g fat and 1.45%/g protein<sup>3</sup>. In our case, the overall adjustment factor was 0.9312, which resulted in an adjusted GI of 78.3 for the white bread control. The differences observed in GI and the other glucose parameters in our study between the white bread control and the breads containing OBC

can therefore not be explained by the small differences in macronutrient composition among these breads.

**Table 2:** Nutrient content and composition of test foods<sup>a</sup>

	Specific volume (mL/g)	Serving size (g)	Amount available carbohydrate <sup>b</sup>	Total dietary fiber	Resistant starch	Beta-glucan
White bread	3.6	53	26.0	1.4	0.25	0.1
Barley bread	2.3	59	26.0	2.3	0.34	0.8
lowOBCB	2.8	73	26.4	4.1	0.46	1.7
degradedOBCB	nd	101	26.2	8.0	0.47	3.8
optimalOBCB	2.1	102	26.3	7.6	0.46	3.8
Lompe	nd	70	26.2	2.8	0.68	0.1
	Protein	Fat	Ash	Water	Energy (kcal)	Energy density (kcal/g)
White bread	4.9	1.2	0.70	18.8	128	2.4
Barley bread	4.7	1.2	0.79	23.7	129	2.2
lowOBCB	6.6	2.3	1.17	32.8	152	2.1
degradedOBCB	9.1	4.1	1.84	51.0	185	1.8
optimalOBCB	8.7	3.7	1.75	53.3	180	1.8
Lompe	4.2	0.6	1.12	34.4	125	1.8

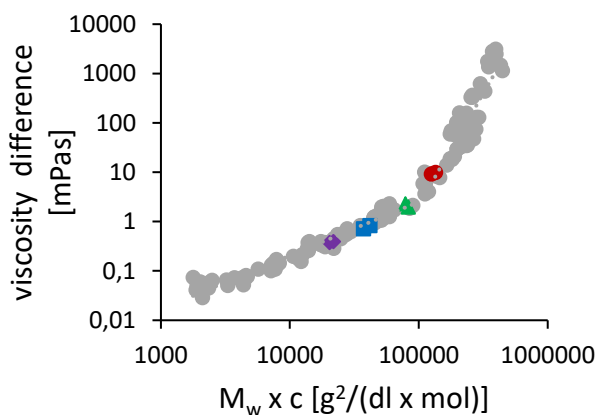
<sup>a</sup> Data are in g per serving if not otherwise stated

<sup>b</sup> Available carbohydrate was calculated from the measured amount of available starch using a conversion factor of 1.1<sup>4</sup>

### Beta-glucan dose and physicochemical properties

The different test foods were subjected to an *in vitro* digestion procedure and the beta-glucan  $M_w$ , solubility and contribution to viscosity (viscosity difference before and after the addition of lichenase) was measured in the extracts (Table 3). As expected, the long proving time of the degradedOBCB resulted in degradation of the beta-glucan by endogenous flour enzymes<sup>23</sup>, while the shorter processes used for optimalOBCB, lowOBCB and barley bread better retained the beta-glucan  $M_w$ . The beta-glucan  $M_w$  was highest for optimalOBCB (592 kDa), followed by lowOBCB (421 kDa) and barley bread (376 kDa) and lowest for degradedOBCB (282 kDa). Interestingly, not only the beta-glucan  $M_w$ , but also the extractability of beta-glucan varied considerably between the breads. The long bread making process of degradedOBCB did not only result in a lower  $M_w$ , but also a much lower fraction of the beta-glucan in the product was

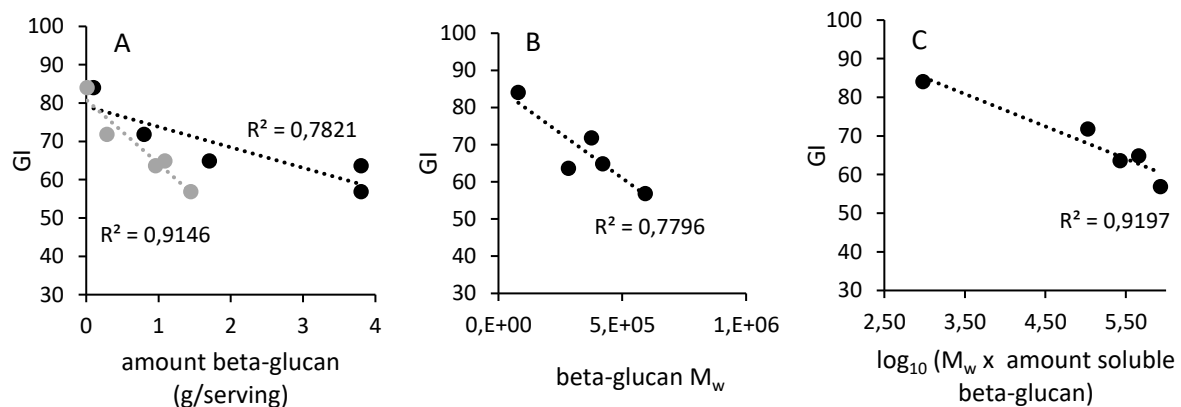
solubilized during the *in vitro* digestion (Table 3). The amount of soluble beta-glucan per serving was consequently considerably lower for degradedOBCB than for optimalOBCB even though the two breads contained the same amount of total beta-glucan. The viscosity of the extracts varied among the breads, but was generally quite low (Table 3). Only the optimalOBCB resulted in extract viscosities above 10mPas (10.6 mPas). OptimalOBCB was therefore the only bread that located in the region above coil overlap in a double logarithmic plot of extract viscosity against the product of beta-glucan  $M_w$  and concentration (Figure 2). As described in previous work, coil overlap has been suggested as a criteria for predicting significant *in vivo* effects on the reduction of postprandial blood glucose levels <sup>26</sup>. However, despite their lower viscosities after *in vitro* digestion, also degradedOBCB and to some extent lowOBCB significantly reduced GI, iAUC and PBGR compared to the white bread control (Table 1).



**Figure 2:** Double logarithmic plot of viscosity difference (before and after lichenase) against the product of beta-glucan concentration and weight average molecular weight of extracts after *in vitro* digestion. Grey circles represent data from a previous study <sup>26</sup>. Data from the present study are in colour: optimalOBCB (red circles), degradedOBCB (blue squares), lowOBCB (green triangles) and barley bread (purple diamonds).

Unlike many previous studies on oat bran muffins <sup>20,22</sup>, extruded cereals <sup>19</sup> and baked granola <sup>21</sup> treated to vary in beta-glucan solubility or MW, no significant correlations between viscosity or  $\log_{10}$  viscosity and any of the glucose variables (GI, iAUC, PBGR) was found. This might be related to the broad range of different beta-glucan doses used (from 1 to 4.8 g per 30 g

available carbohydrate) in our study, while many of the aforementioned studies compared the effect of varying beta-glucan characteristics at the same beta-glucan dose. The absence of a significant correlation between viscosity after *in vitro* digestion and the glycaemic response to the different breads points towards a less dominant role of bulk viscosity as previously suggested<sup>38</sup>. This is in agreement with recent suggestions, that the viscosity increase that can be expected in the intestinal lumen after the consumption of foods rich in soluble dietary fiber is unlikely to be high enough to substantially delay the diffusion of glucose<sup>14</sup>. However, the physicochemical properties of beta-glucan still seem to be important for its ability to attenuate glycaemic response. The amount of soluble beta-glucan per serving gave a better correlation with GI than the total amount of beta-glucan per serving (figure 3a). Beta-glucan  $M_w$  was negatively correlated with GI (figure 3b) and  $\log_{10}(M_w \times \text{amount of soluble beta-glucan per serving})$  gave the best correlation with GI among all the different tested variables of beta-glucan physicochemical characteristics (figure 3c).



**Figure 3:** Correlation between GI and A: amount beta-glucan per serving (black: total beta-glucan; grey: soluble beta-glucan), B: beta-glucan  $M_w$ , C:  $\log_{10}(M_w \times \text{amount soluble beta-glucan per serving})$ .

Beta-glucan has been reported to interact with the intestinal mucus layer, thereby increasing its barrier function to lipid digestion products<sup>39</sup>. If such an interaction may also play a role for the diffusion of starch digestion products remains to be seen. However, it seems plausible that only the soluble/extractable fraction of beta-glucan in a food product interacts with the mucus layer,



which would explain the dominant effect of soluble beta-glucan per serving for our bread products.

Interaction of beta-glucan with the mucus layer may require coil overlap, which means that the dissolved beta-glucan molecules get entangled (with each other or with other macromolecules) due to their size (MW) and concentration. The occurrence of coil overlap after *in vitro* digestion has been previously shown to correlate well the ability of different beta-glucan containing food products to reduce glycaemic responses <sup>26</sup>. However, the concentration of beta-glucan at the mucus layer may be different than in the lumen. Additionally, the digestive system may adjust the volume of the meal and equilibrate viscosity by increasing the secretion of gastric fluids <sup>40</sup>. Interestingly, increasing the viscosity of a glucose and beta-glucan solution by reducing the solution volume had no effect on the glycaemic response, while increased viscosities brought about by higher beta-glucan MW or dose clearly reduced the glycaemic response <sup>41</sup>.

Despite the tendencies seen in our study and the numerous studies demonstrating the importance of beta-glucan solubility and MW for their reduction of glycaemic responses <sup>19-21, 42</sup>, there was no significant difference in iAUC, GI or PBGR between the optimalOBCB and the degradedOBCB (Table 1). Even though the latter had a significantly lower beta-glucan  $M_w$  and solubility (Table 3). Both breads were formulated to fulfill the EFSA criteria for the health claim on lowering of post-prandial glycaemic response of 4 g beta-glucan per 30 g available carbohydrate. This is a very high dose of beta-glucan, which is difficult to achieve in bread and requires the use of special milling fractions with elevated levels of beta-glucan instead of regular oat or barley flour. The resulting doughs have a very high water binding capacity and the serving size of the two high dose OBC breads was twice as much as for the white bread (Table 2). In fact, at 50 g available carbohydrate, the serving size was too big to be consumed within 15min and all the test foods were therefore downscaled to 25 g available carbohydrate. Portion size was the variable which correlated best with PBGR (Pearson correlation coefficient:

-0.963,  $p = 0.008$ ). The high portion size of optimalOBCB and degradedOBCB may therefore have resulted in a slower appearance of starch into the small intestine, since boluses from these two breads released from the stomach would contain less starch compared to an equal bolus of white bread.

**Table 3:** Physicochemical properties of test foods <sup>a</sup>

	Soluble beta-glucan in % of total	Beta-glucan $M_w$ (kDa)	Viscosity of extract (mPas)	Amount soluble beta-glucan per serving (g) <sup>b</sup>	Amount beta-glucan (g) per 30 g available carbohydrate <sup>c</sup>	RDS in % of total starch
<b>White bread</b>		80 ± 7	1.2 ± 0.01	0.01	0.1	66.5 ± 2.5
<b>Barley bread</b>	35.5 ± 3.3	376 ± 16	1.6 ± 0.01	0.27	1.0	60.5 ± 1.3
<b>lowOBCB</b>	63.9 ± 1.1	421 ± 23	3.2 ± 0.3	1.06	2.1	60.9 ± 7.1
<b>degradedOBCB</b>	25.2 ± 0.1	282 ± 23	1.8 ± 0.1	0.96	4.8	68.0 ± 3.8
<b>optimalOBCB</b>	38.4 ± 1.7	592 ± 5	10.6 ± 0.5	1.46	4.8	62.4 ± 5.2
<b>Lompe</b>	nd	nd	nd	nd	0.1	46.3 ± 2.3

<sup>a</sup> values are averages ± standard deviations if not otherwise stated.

<sup>b</sup> all standard deviations below 0.006

<sup>c</sup> all standard deviations below 0.02

### ***In vitro* starch digestibility**

The biggest difference in the rate and extent of starch digestion was seen between lompe and the bread products, while there were only minor differences among the different breads (data not shown). The content of rapidly digestible starch (RDS), which is the proportion of starch digested during the first 20 min of the *in vitro* digestion, was similar among the breads and ranged from 60.5 to 68%, while lompe had a RDS content of 46.3% (Table 3). The rate of *in vitro* starch digestibility in cereal products has been shown to correlate with the GI of the products <sup>2</sup>. The lower glycaemic response to lompe compared to white bread seen in this study might therefore be due to the low content of RDS in this product. The difference in glycaemic response between white bread and the breads containing OBC observed in this study can, however, not be explained by any difference in starch digestibility. This is in contrast to findings for baked oat granola, where high beta-glucan MW and high beta-glucan to starch ratios

resulted in increasingly reduced levels of RDS alongside with a lower PBGR and iAUC <sup>21</sup>. Among others, viscosity mediated reduced enzymatic accessibility of starch and reduced availability of water for starch gelatinization and hydrolysis have been proposed as potential mechanisms by which soluble dietary fibers such as beta-glucan may reduce starch digestibility <sup>14, 43</sup>. The water content of the breads prepared with OBC in our study was very high (45 to 52%), while the baked granola had a water content of 40% <sup>21</sup>, which may explain the absence of any effect on *in vitro* starch digestibility with increased amounts and M<sub>w</sub> of beta-glucan in the breads.

### **Conclusions and future perspective**

At the high dose of 4 g beta-glucan per 30 g available carbohydrate, even breads with processing-induced reductions of beta-glucan M<sub>w</sub> and solubility, significantly lowered PBGR, iAUC and GI compared to white bread. This might be positive, as physicochemical properties of beta-glucan are not included in the EFSA health claim definition on post-prandial blood glucose. However, the high dose that is required is very difficult to achieve in bread, which limits the use of the claim. Here we show that nearly the same effect could be achieved with half the beta-glucan dose if the process is optimized to maximize beta-glucan MW and solubility. There was no significant correlation between the viscosity after *in vitro* digestion and any of the *in vivo* glucose variables. *In vitro* digestion can nevertheless give useful information on the potential of beta-glucan containing food products to reduce post-prandial glycaemic responses for example by giving information on the solubility of beta-glucan under physiological conditions as we found a significant inverse correlation between the total amount of soluble beta-glucan per serving and GI. Further studies are needed to elucidate the mechanisms of action of cereal beta-glucan, which include nutrient dilution, reduced gastric emptying, reduced starch digestibility and reduced diffusion of starch degradation products due to locally increased viscosity e.g. at the mucus layer. Apart from beta-glucan dose, MW and

solubility, also the food matrix (for example the water content) may influence the efficacy and mechanism of action of beta-glucan containing food products. More information is needed before “cut off” values for beta-glucan MW and solubility that may ensure significant effects at lower doses than the current claim can be defined. Nevertheless, a future definition of such “cut off” values might help to ensure the efficacy of products bearing the claim and at the same time enable a reduction of the required dose, thereby increasing the number of food products bearing it. Typical low GI breads are often pumpnickel style breads with whole kernels or breads with high levels of organic acids, which not all consumers like. Breads containing high enough amounts of cereal beta-glucans with the right physicochemical properties or the Norwegian “lompe” may therefore be good alternatives for filling the low GI bread gap.

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### **Conflicts of Interest**

There are no conflicts of interest to declare.

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