THE GLYCEMIC RESPONSE ELICITED BY OAT β-GLUCAN SOLUTIONS AND HARD GEL VARYING IN PHYSIOCHEMICAL PROPERTIES AND FOOD FORM

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
Department of Nutritional Sciences
University of Toronto

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Master of Science
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Abstract

The ability of the soluble fibre \((1\rightarrow3)(1\rightarrow4)\)-β-D-glucan to attenuate postprandial glycemic responses depends on its viscosity which, in turn, depends on molecular weight (MW) and dose. However, the effect of altering viscosity by changing solution volume is unknown. Furthermore, β-glucan solutions may form hard gels when left to age, but the effect of these gels on glycemic responses is unknown. Therefore, the effects of varying the MW and volume of β-glucan solutions and hard gels, on glycemic responses were determined. The results showed that glycemic responses were reduced by increasing viscosity by increasing MW but not by reducing solution volume. Although β-glucan gels reduced the rate of glucose diffusion \textit{in vitro}, they had no effect on glycemic responses \textit{in vivo}. Thus, changing solution viscosity through changes in volume does not alter the effect of β-glucan on glycemic response, and β-glucan gels are ineffective at attenuating \textit{in vivo} glycemic responses.
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<td>130H</td>
<td>High molecular weight β-glucan solution, 130ml in volume</td>
</tr>
<tr>
<td>130L</td>
<td>Low molecular weight β-glucan solution, 130ml in volume</td>
</tr>
<tr>
<td>130N</td>
<td>Control glucose solution, 130ml in volume</td>
</tr>
<tr>
<td>250H</td>
<td>High molecular weight β-glucan solution, 250ml in volume</td>
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<td>Low molecular weight β-glucan solution, 600ml in volume</td>
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<tr>
<td>600N</td>
<td>Control glucose solution, 600ml in volume</td>
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<tr>
<td>LMWG</td>
<td>Low molecular weight β-glucan hard gel</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Incremental area under the glycemic response curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index (kg/m²)</td>
</tr>
<tr>
<td>C</td>
<td>Concentration (g/mol)</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of Polymerization (of β-glucan polymer)</td>
</tr>
<tr>
<td>GOPOD</td>
<td>Glucose Oxidase/Peroxidase</td>
</tr>
<tr>
<td>HPSEC</td>
<td>High Performance Size-Exclusion Chromatography</td>
</tr>
<tr>
<td>MW</td>
<td>Weight Average Molecular Weight</td>
</tr>
<tr>
<td>RDS</td>
<td>Rapidly Digestible Starch</td>
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SDS  Slowly Digestible Starch
SD   Standard Deviation
SEE  Standard Error of the Estimate
SEM  Standard Error of the Mean
T2D  Type 2 Diabetes; Type 2 Diabetic
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INTRODUCTION
INTRODUCTION

Over 200 million people worldwide are affected by diabetes, a disease characterized by the inability to produce or utilize insulin (1). This condition results in elevated blood glucose levels (hyperglycemia), and may lead to serious complications including heart disease and premature death. There is no known cure for diabetes, but measures can be taken to manage this disease or help prevent its onset.

Preventing hyperglycemia by lowering the glycemic response after meals has been associated with a reduced risk of diabetes (3, 4). This has been shown with the drug acarbose, which inhibits enzymes in the small intestine from hydrolyzing carbohydrate polymers to glucose. Glucose absorption is then delayed and the subsequent rise in glycemic response is attenuated. However, a diet high in fibre has also been associated with a lowered risk of diabetes, which can similarly be attributed to preventing hyperglycemia (3, 4).

One type of dietary fibre that lowers glycemic response is (1\(\rightarrow\)3)(1\(\rightarrow\)4)-\(\beta\)-D-glucan, which occurs naturally in cereal grains including oats and barley (5). The (1\(\rightarrow\)3) glycosidic bonds of the polysaccharide allow \(\beta\)-glucan polymers to be soluble and form viscous liquids in solution. Furthermore, these solutions may form solid hard gels when aged. It is believed that viscous \(\beta\)-glucan solutions trap carbohydrates that are released from food digestion in the gut, which would slow the absorption of carbohydrates available for uptake and attenuate the subsequent glycemic response (5). Thus, \(\beta\)-glucan in oat-containing foods may serve as a useful food alternative to drugs for diabetes prevention. Furthermore, \(\beta\)-glucan extracts are commonly used in food applications and may be used in developing novel food products that confer the benefit of blood glucose-lowering.
The ability for β-glucan solutions to lower glycemic response increases with liquid viscosity, a property dependent on the molecular weight of the β-glucan polymer (MW) and the solubility or concentration (C) of the polymers in solution (6). When MW and C are high, glycemic response is most effectively lowered. This was demonstrated in a study by Wood et al. (6), who tested the effect of β-glucan solutions of various viscosities on glycemic response. In this study, viscosity was altered by changing the MW and C of β-glucan in solution, while keeping the solution volume constant. When solutions containing successively greater doses of high MW β-glucan (~800,000g/mol) were administered to healthy subjects, the magnitude of the blood glucose-lowering effect increased successively. This firstly illustrated a dose-response relationship between β-glucan and the glycemic response-lowering effect. Secondly, since increasing β-glucan dose also increases solution viscosity, the experiment showed that viscosity was directly related to the lowering of blood sugar levels (6). The importance of high viscosity was further demonstrated when the experiment was repeated using a lower-MW β-glucan (~200,000g/mol) which produced low solution viscosity and yielded a weaker response-lowering effect. Thus, the study established that increasing the dose or concentration, C, of β-glucan in solution and increasing β-glucan MW can increase solution viscosity, and that increasing viscosity leads to a greater attenuation of glycemic response (6, 7).

Achieving changes in viscosity, however, is not limited to varying β-glucan MW or dose. This is true because while viscosity is dependent on MW and C, C is itself dependent on dose and the volume of solution in which β-glucan is dissolved. Thus, viscosity can be altered by changing the solution volume. Yet the effect of β-glucan viscous solutions on glycemic response where viscosity is altered by volume remains to be investigated.
The glycemic response-lowering effect of β-glucans has also been demonstrated in the food matrix. Studies have shown that adding β-glucan to carbohydrate-containing foods can lower the expected glycemic response that the food alone would normally elicit, in both Type 2 diabetic subjects (8) and healthy subjects (9). Furthermore, the addition of β-glucan to carbohydrate foods can lower its glycemic index value (GI). The GI measures the potential for the available carbohydrate in a food to raise blood sugar levels compared to an equivalent amount of glucose (10). Jenkins et al. (8) showed that an oat bran bar enriched with β-glucan elicited a greater lowering effect than a commercial oat bran cereal containing 50% less β-glucan. Furthermore, by examining the reduction in GI values achieved in other studies where β-glucan was added to carbohydrate-containing foods, it was determined that a 3.8 GI unit-reduction for a food is attained for every gram of β-glucan added (8). These findings not only demonstrate that β-glucan is effective in the food matrix, but also support the dose-response relationship between β-glucan and glycemic response-lowering.

A study by Tosh et al. (9) examined the effects of high to low MW β-glucan in muffins (~130,000 – ~2,000,000 g/mol respectively). As expected, muffins containing high MW β-glucan had a greater glycemic response-lowering effect than muffins containing low MW β-glucan. However, β-glucan solubility increases as MW decreases, which means that lowering MW β-glucan can increase the concentration of polymers in solution and perhaps strengthen glycemic response-lowering. Nevertheless, solubility was lost when MW was very low. This can be explained in the context of gelation phenomenon which is unique to low MW β-glucans. Although low MW solutions are less viscous than high MW solutions, it is the low MW solutions that tend to form strong gel networks when left to age. This is because low MW polymers are less stearically hindered compared to their high MW counterparts,
allowing low MW polymers to more easily associate with one another and more efficiently form junction points that make up a gel network (2). However, under the experimental conditions of this study, a lack of water hindered polymer association and instead led to the formation of insoluble polymer aggregates (9). As a result, the solubility of the lowest MW β-glucan was very low and gave a solution of very low viscosity incapable of gel formation. While the lowest MW polymers had a weak lowering effect in this study, the findings raise the question of what role low MW β-glucan gels themselves may have in lowering blood sugar levels when these gels are properly formed. Since liquid viscosity increases with increasing MW but it is the low MW β-glucans which are associated with gel formation, differences in the physical properties between gels and liquids may translate to differences in their effect on glycemic response. However, no studies have directly tested the effect of β-glucan gels on blood sugar levels.
Two gaps in knowledge regarding how the structure and physical form of β-glucan relates to its function of lowering glycemic response have been identified. Therefore, a study consisting of two experiments was conducted to fill these voids:

**Experiment 1**

The effect of viscosity achieved through changes in volume is unknown. Thus, the effects of β-glucan on glycemic response were examined when solution volume was altered and the β-glucan dose was held constant. We hypothesized that the blood glucose-lowering effect would decrease as the solution volume increased. Our hypothesis was tested at two levels of viscosity by using high MW β-glucan polymers (high viscosity level), as well as low MW β-glucan polymers (low viscosity level).

**Experiment 2**

Since studies have focused on the importance of liquid viscosity but not the potential of β-glucan hard gels on lowering blood sugar levels, our study also tested the hypothesis that β-glucan gels would have an effect on glycemic response, and that this effect may parallel that of β-glucan solutions.

By performing the two proposed experiments, we aimed to determine the effects of β-glucan solution volume on blood glucose levels, and sought to identify what food forms of β-glucan are appropriate for lowering glycemic response. In doing so, we not only worked to fill the gaps in knowledge that exist on the structure-function relationship of β-glucan fibre, but also helped in the development of β-glucan food products that assist individuals with glycemic control.
CHAPTER 1

REVIEW OF THE LITERATURE
1.1 GENERAL PROPERTIES OF β-GLUCAN

1.1.1. Basic Molecular Structure and Characteristics

\[(1\rightarrow3)\ (1\rightarrow4)\ β-D-glucan\ (\text{referred to as} \ β-glucan \text{ herein})\ is \ a \ soluble \ dietary \ fibre \ that \ is \ naturally \ found \ in \ cereal \ grains \ including \ oats, \ barley, \ wheat, \ and \ rye, \ as \ well \ as \ in \ lichen \ moss \ (5). \ The \ β-glucan \ polymer \ is \ described \ as \ a \ mixed-linkage \ polysaccharide \ which \ consists \ of \ cellulose-like \ units \ joined \ together \ by \ (1\rightarrow3)-\text{glycosidic \ linkages (Figure 1.1).} \]

Whereas cellulose comprises strictly of \((1\rightarrow4)\)-linked glucose monomers and is therefore an insoluble straight-chain polysaccharide, the \((1\rightarrow3)\) linkages in β-glucan disrupt the linearity of the polymer and make it soluble in solution. (5). The number of consecutive \((1\rightarrow4)\)-linked glucose monomers within a cellulose-like unit, (referred to as the degree of polymerization (DP) of that unit,) may vary between 2 and over 15 monomers (11). However, units containing three (DP3) and four monomers (DP4) are most prevalent in the cereal β-glucans. In oats, DP3 and DP4 units together make up over 90% of the β-glucan polymer (11, 12). Furthermore, the relative abundance of DP3 and DP4 units varies depending on the β-glucan source, thus allowing for individual sources of β-glucan to be identified by a unique DP3:DP4 ratio (11, 13).
The structural features of β-glucan are vitally important for the unique rheological properties of the polymer, which in turn affect how β-glucan confers physiological health benefits when consumed. For instance, the combination of (1→3) and (1→4) linkages allows for β-glucan to form viscous solutions when solubilized, and the DP3:DP4 ratio is not only a molecular fingerprint that distinguishes β-glucan sources, but is also a factor in the ability for β-glucan solutions to form solid hard gels (14). Moreover, the polymer size or molecular weight is a crucial factor in the behaviour and formation of β-glucan solutions and gels. Since the physiochemical properties of β-glucan depend upon the rheological properties of the polysaccharide, it is important to discuss how β-glucan structure, rheology, and health effects are related to one another.

1.1.2. **Molecular Weight**

The rheological properties of β-glucan are directly affected by polymer size, and it is therefore necessary to report molecular weight (MW) when characterizing β-glucans. The polydisperse nature of polysaccharides means that β-glucan MW is not based on a single
value but rather a range or average of MW values (14). High-performance size exclusion chromatography (HPSEC) has allowed for the MW of β-glucan in both the native grain and in food products to be determined quickly and efficiently. The MW values for oat β-glucan that are reported in the literature vary widely from $6.5 \times 10^4$ – $3.1 \times 10^6$ g/mol (15, 16). The apparent discrepancy in values is explained by the numerous factors which may affect polymer MW, ranging from the growing environment and genetics of the crop to the effects of processing and storage on β-glucan when it is incorporated into food products. Because of the importance of MW for β-glucan bioactivity, a detailed discussion on the factors affecting MW will follow (section 1.3), and will also be discussed in the context of the consequences on glycemic response (section 1.5).

1.2 RHEOLOGICAL PROPERTIES OF β-GLUCAN

β-glucan is able to form viscous liquids when solubilized in solution and may form solid hard gels when those liquids are left to age. Using experimental techniques in rheology, studies on the behaviour of β-glucan solutions and gels have revealed how polymer structure and chain interaction give rise to their rheological properties. Such techniques include applying steady shear force to polymer solutions under constant temperature, which allows for the measurement of apparent solution viscosity. Apparent viscosity is measured over a range of frequencies of the applied force (17, 18). Another technique involves subjecting solutions and gels to a constant oscillatory force over a range of frequencies in order to obtain viscoelastic measurements (information on the liquid-like and solid-like behaviours of a substance (17-19). In both cases, the viscosity and viscoelastic values obtained at a single
frequency may be used to compare the rheological properties of the substances being measured.

1.2.1. Viscosity

The viscosity of a $\beta$-glucan solution depends on the dose or concentration (C) and the molecular weight (MW) of $\beta$-glucan polymers in solution (6). As C and MW increase, the entanglement of polymer chains also increase, giving rise to higher solution viscosity (16, 20). The sensitivity of solution viscosity to changes in MW and C has been demonstrated. Above a critical C of 0.3% (the concentration at which polymer chains interact), the viscosity of a high MW $\beta$-glucan solution increases exponentially (5, 21), while a 23% drop in MW has been shown to cause a 73% loss in solution viscosity for a given C (22). In fact, Wood et al. (6, 7) have demonstrated that a linear relationship exists between the log (viscosity) of a $\beta$-glucan solution and the log (C x MW) of dissolved polymers. Therefore, both C and MW may be manipulated to alter solution viscosity. Moreover, low solution viscosity due to low polymer C can be overcome by increasing polymer MW (20).

The apparent viscosity of a high MW $\beta$-glucan solution typically remains constant at low shear rate but decreases as shear rate increases (5, 14, 23). This is because when a low shear rate is applied, polymer associations that are disrupted by shear force are given sufficient time to re-form, allowing the viscosity level to be conserved. However, the loss in polymer entanglement at higher shear rates is not recovered, resulting in a reduction in viscosity known as shear-thinning behaviour (24, 25). A low MW $\beta$-glucan solution behaves similarly to high MW solutions at high shear rates, but as shear rate decreases to low levels, low MW solutions exhibit weak shear-thickening behaviour. The ability for the viscosity of a
low MW solution to increase as shear rate weakens has implications for how β-glucan solutions form gels.

1.2.2. Gel Formation

When a β-glucan solution is left at rest, it may solidify to form a solid hard gel over time. Such a gel is described as a substance that does not flow as a liquid does and retains the shape of the container that holds it (2, 25). In order for a β-glucan gel to form, the solubilized polymers must associate with one another at junction zones along their polysaccharide chains, where they are joined by intermolecular forces in the creation of a molecular gel network.

The successful formation of a gel can be verified by taking the viscoelastic measurements of the gel, namely the storage modulus (G’) and the loss modulus (G’’) (25). For a given substance, regardless of whether the substance is a solid or a liquid, G’ and G’’ represent the solid-like and liquid-like behaviours of that substance respectively (18). When oscillatory force is applied at increasing frequency to a β-glucan solution of zero to very low viscosity, G’’>G’ is observed throughout the period of applied force, indicating that the substance is fluid and exhibits no solid-like behaviour. For a solution of high viscosity, G’’>G’ is observed at the beginning of the period where the frequency of the force applied is low, but as the frequency increases, G’ also increases and eventually overtakes G’’. The more viscous the solution is, the lower the frequency at which G’>G’’. Conversely, when the force is applied to a true, solid gel, G’>G’’ is observed throughout the measurement period, indicating that the substance has no liquid-like behaviour (23).

Similar to the development of viscosity, the ability for β-glucans to form gels is dependent on the MW of the polymer (26). Low MW solutions may form gels in the order of
hours, while it has been shown that high MW solutions of the same concentration do not solidify even after aging for 7 days (2, 25, 27). In a gel curing experiment by Lazaridou et al., oscillatory force was applied at a constant frequency to β-glucan solutions of high to low MW for several days in order to track the changes in G’ and G’’ of each solution (27). At the beginning of the experiment, all solutions exhibited G’’>G’, which reflected the liquid state of the solutions, but over time G’ rose and surpassed G’’ to reach a plateau value called G’ max. Arrival at this value indicates that the maximum number of intermolecular cross-links between β-glucan polymers have formed, and that the β-glucan solution has gelled (24). For low MW solutions, G’ max was reached much earlier in the experimental period than high MW solutions, proving that gelation is achieved at a faster rate with low MW polymers. Furthermore, the magnitude of G’ max is a measure of the connectivity of the gel network (25), and the G’ max values were higher for low MW gels than high MW gels. Thus, the low MW gels not only formed more efficiently, but also possessed a stronger gel network. However, additional forms of rheological measurements revealed that high MW gels were physically more firm and less brittle than low MW gels. The reason for the discordance between the strength of the gel network and the overall physical strength of the gel itself is unknown (27). Nevertheless, it appears that a trade-off exists for high MW β-glucans, where greater physical gel strength compensates for a slow gelation rate.

The tendency for low MW solutions to gel is explained by the lower molecular size of the polymers. When molecular size is low, there is less stearic hindrance preventing polymers from interacting with one another and an increased mobility for polymers to associate. This makes the formation of junction zones more efficient and the polymer associations stronger than those made between high MW polymers. Thus, when a low
frequency shear force is applied to a low MW solution, viscosity increases as the shear rate decreases because the efficient formation of polymer interactions overcomes the applied force (21).

However, since the success of gel network formation depends on the interaction between polymers, it is not only important to consider the degree of stearic hindrance that prevents polymer association, but also the actual site of polymer association. For oat β-glucan, two theories have been proposed as to the location of these sites (25). The first suggests that adjacent polymers interact at long cellulose-like regions (DP6-DP9 in length) through hydrogen bonding (Figure 1.2 (a)). The second theory suggests that gel formation in oat β-glucan is similar to that of lichen moss, where polymer interaction occurs at regions of consecutive DP3 units linked by (1→3) glycosidic bonds (Figure 1.2 (b)). Further evidence supporting the latter theory comes from studies which found that β-glucan solutions with a high DP3:DP4 ratio gel more efficiently (2, 23). Cui et al. (28) found that β-glucan isolated from wheat gelled faster than either oat or barley β-glucan, which corresponds to the higher DP3 (and lower DP4) content of wheat β-glucan compared to the other two grains.
Figure 1.2  Theoretical sites of junction zone formation between oat β-glucan polymers.

Figure 1.3  The β-glucan polymer and target sites for various hydrolysis methods. Both cellulase and lichenase hydrolyze β-(1→4) linkages. Hydrochloric acid targets both β-(1→3) linkage and β-(1→4) linkages.
In order to determine which theory is correct, experiments involving polymer hydrolysis have been performed on β-glucan used to form gels. In enzyme hydrolysis, cellulase cleaves the (1→4) bonds in β-glucan when there are two consecutive (1→4) linkages (Figure 1.3). This thus reduces cellulose-like regions of the polymer. Lichenase also cleaves (1→4) bonds, but only when an adjacent (1→3) linkage is present. This means that lichenase reduces the abundance of (1→3)-linked DP3 units while leaving long stretches of cellulose-like regions intact. Hydrolysis with hydrochloric acid breaks both (1→3) and (1→4) bonds, but whether one type of bond is preferentially disrupted is unknown.

In a study by Tosh et al., low MW β-glucan was treated with lichenase, cellulase, or hydrochloric acid prior to being used to form gels (25). The strength of the gels was found to be associated with the treatment type, following the order of cellulase>acid>lichenase. Since β-glucan hydrolysis with lichenase produced the weakest gel, the results showed that the destruction of consecutive DP3 units was detrimental to gel strength, and that these sequences of repeating DP3 played an important role in gelation. Tosh et al. found further evidence to support the DP3 theory by relating the abundance of DP3 units in β-glucan from different sources to the strength of the gels formed by those polymers (2). In order of decreasing DP3 content, the sources used were lichen>wheat>barley>rye>oat. Gel strength was positively correlated with DP3 content and not with the abundance of cellulose-like regions, indicating that the regions of repeating DP3 units are important for gel formation. Furthermore, since the number of consecutive (1→3)-linked DP3 units in a region increases with increasing total DP3 content (28), gel strength depends on both the abundance and length of these regions.
It is clear that both MW and DP of β-glucan polymers affect the rheological characteristics of the polysaccharide. The DP3:DP4 ratio of β-glucans varies between botanical sources and the values found in the literature for the different sources are relatively consistent compared to the reporting of MW values. Given the important role that MW plays in dictating β-glucan rheology, it is important to discuss the potential factors which affect MW values.

1.3 MOLECULAR WEIGHT OF β-GLUCAN

1.3.1. Factors Influencing Molecular Weight

In the native cereal grain, both genetics and environment have been shown to influence β-glucan MW. Papageorgiou et al. (29) found that differences in β-glucan MW of several unique oat varieties were dependent on cultivar rather than environment, since the oat varieties studied were grown under identical environmental conditions. In contrast, other studies have shown that oat varieties which were grown in locations and years of the lowest annual rainfall achieved the highest β-glucan MW (30, 31). However, interaction effects of cultivar and environment on MW have also been reported, meaning that both of these factors are likely to be important determinants of MW (31).

Differences in reported MW may also arise from differences in method for extracting β-glucan from the native grain (32). For instance, Beer et al. (33) extracted over 70% β-glucan from oats using hot water without compromising MW, while an alkali solvent extracted 89-100% β-glucan but reduced the MW. Rimsten et al. (34) also found that alkali extraction of β-glucan from oats gave higher β-glucan yield but lower MW compared to hot water extraction. However, the MW of extracted β-glucan in barley, barley food products and
oat food products did not differ greatly whether hot water or alkali solvents were used. Thus, not only does extraction method give different MW values, but the effects of extraction may vary between unprocessed grains and processed foods.

β-glucanases which degrade β-glucan polymers also lower MW (34). These endogenous enzymes are found in the native grain and must be deactivated prior to β-glucan extraction. The presence of β-glucanases is especially important to consider when making oat-based foods such as yeast-leavened breads and muffins made with oat bran, since β-glucanases may remain active during the preparation of such foods. Andersson et al. showed that the β-glucan MW of oat breads (35) and rye crisp breads (36) decreased as fermentation time increased, suggesting that a longer preparation time prolonged β-glucanase activity. Similarly, Tosh et al. (37) found that β-glucan MW was lower in oat foods involving a greater preparation time before cooking (bread dough which required kneading and fermenting; pasta made with an extruder) compared to foods requiring less preparation time (oat granola and porridge which only required mixing before being cooked at 350°C for 20 minutes and 100°C for 5 minutes respectively). The loss in MW was attributed to enzymatic hydrolysis of the polymers.

The fact that high cooking temperatures did not decrease the MW of oat foods shows that β-glucan polymers can withstand high temperatures without depolymerisation, and this has been observed in other studies (35, 38). Although one study (39) did find that cooking oat bran muffins halved the β-glucan MW compared to that of the original oat bran, the depolymerisation may have been due to the presence of β-glucanases in the batter rather than exposure to heat. In another study by Tosh et al. (38), two β-glucan extracts with MW of 70,000g/mol and 40,000g/mol respectively were solubilized at 60, 70, 80 and 90°C for three
hours. HSPEC revealed that for both extracts, MW was the same irrespective of temperature treatment. The results show that even when exposed to high temperatures for several hours, β-glucan depolymerisation does not occur. In addition, the high temperatures involved in commercial extrusion processes for making oat bran cereal had no effect on β-glucan MW (37). Freezing temperatures also do not appear to affect MW, as the frozen storage of β-glucan extracts actually prevented MW from decreasing (22). It has also been shown that the frozen storage of oat bran muffins has minimal effects on MW (40). However, MW is reduced when oat bran muffins are subjected to repeated cycles of freezing and thawing (41).

β-glucan polymers are sensitive to acidic conditions and may depolymerize when exposed to strong acids. This is an important factor to consider in both liquid and solid foods containing β-glucan. In the study by Andersson et al. (35), when oat bran was used to make yeast-leavened breads, β-glucan MW decreased with increasing fermentation time. Although the polymer degradation was mainly due to enzyme activity, lactic acid produced by fermenting yeast was thought to contribute to the loss in MW through acid hydrolysis. Kivela et al. (42) has shown that ascorbic acid, a common food additive and preservative in food products, may act as a strong oxidative agent that significantly reduces the MW and viscosity of β-glucan drinks. In contrast, citric acid, another common food additive, did not affect β-glucan MW. Nevertheless, it is necessary to take note of the conditions under which β-glucan MW may be affected.

The factors discussed influence β-glucan MW by affecting the actual polymer size. However, the method by which MW is measured is equally important for determining MW value in practical settings. The most reliable and commonly used technique for measuring β-glucan MW when crude extracts are used is HPSEC. This method separates polymers of
different MW by first eluting higher MW β-glucan polymers from the elution column, followed by low MW polymers (12, 22, 39, 43). Calcofluor, a substance which selectively binds β-glucan and fluoresces with an intensity proportional to the amount of β-glucan bound, is used in the columns. This allows for post-column quantification of the amount of β-glucan in eluted fractions of different MW. The clear advantage of HPSEC with Calcofluor binding is that β-glucan extracts do not need to be pure. However, the method requires that the columns first be calibrated against standards of known MW. Pullulan standards have commonly been used because of their lower polydispersity compared to β-glucan standards. However, using pullulan standards gives overestimates of β-glucan MW value. This not only leads to inaccurate measurements but also makes it difficult to compare MW values between studies (5, 22).

For β-glucan extracts of high purity, instruments are available for measuring MW without the need to calibrate the system against multiple standards and without the use of Calcofluor. In such systems, multiple detectors gather different types of information about the properties of the substance being eluted from the column, including light scattering, concentration, and rheological measurements. The combination of various types of information allows for better characterization of the substance and gives a more precise measure of MW (24).

1.3.2. Achieving Target Molecular Weight

Despite the fact that the MW of β-glucans may vary widely, it is possible to achieve specific MW targets. Fractionation techniques can be used to overcome the high polydispersity of β-glucan by using ammonium sulphate ((NH₄)₂SO₄) to selectively precipitate β-glucan from solution (12, 44, 45). At low (NH₄)₂SO₄ concentration, β-glucan
polymers with high MW are the first to be precipitated, forming the first β-glucan fraction. The remaining fractions are obtained by successively increasing the (NH₄)₂SO₄ concentration to obtain polymers of successively lower MW. The MWs of the fractions vary from each other, but the MWs of the polymers within the same fraction are similar. Also, the polymers from different fractions differ only in MW but not molecular structure (44). This technique could be useful in producing β-glucan extracts of high MW, narrow polydispersity, and thus optimal bioactivity.

Hydrolysis techniques using acid and enzyme digests have also been used to attain MW targets that are lower than the native β-glucan MW (25). However, the type of digest may affect the physical and rheological properties of the β-glucan polymers. The enzyme lichenase disrupts continuous runs of DP3 units, whereas cellulase breaks apart long cellulose-like regions. Tosh et al. (25) found that since the consecutively-linked DP3 units are important in gel formation, β-glucan digested with lichenase formed weaker gels than those digested with cellulase.

1.4 SOLUBILITY AND EXTRACTABLILITY OF β-GLUCAN

Another important factor to consider for β-glucan bioactivity is the degree to which it is accessible under digestion, and this is especially true when β-glucan is incorporated into solid foods. Since the proposed mechanism with which β-glucan affects glycemic response depends on viscosity development in the gut, this requires that β-glucan be extractable from the food matrix in order to solubilize in the surrounding aqueous environment. The amount of soluble or extractable β-glucan in foods can be determined by using an in vitro digestion model (14). With the presence of digestive enzymes in the system, the model mimics the in
*vivo* digestion process, and the amount of β-glucan that is released from the food into the surrounding solution can be measured (39).

Several studies using the digestion model have shown the effects of food processing and storage on β-glucan solubility. Employing the model, it has been shown that increased solubility can be achieved by mechanical reduction in the particle size of oats by milling (5, 43). In other studies, heat treatment was shown to increase solubility, as solubility of β-glucan from oat bran cereals increased with increasing extrusion temperature used in the making of the cereals (37), and solubility of β-glucan from oat flakes increased after 10 minutes of boiling (46).

Another way to increase solubility is by reducing MW. This was shown in a study by Tosh *et al.* (9) where the β-glucan MW of oat bran muffins were varied and the solubility of β-glucan was measured. Initially, the solubility of β-glucan measured in the *in vitro* digestion extracts increased with decreasing MW. However, when MW was very low, solubility dramatically decreased. This was due to aggregation phenomenon of low MW polymers. Normally in a β-glucan solution, low MW polymers are highly mobile and interact to form a gel network. In the food matrix of the muffin, a lack of water significantly hampered polymer mobility, which lead not to gel network formation, but the production of insoluble aggregates. β-glucan solubility was then reduced and the viscosity of the digesta was likely compromised. In a separate study by Regand *et al.* (47), oat bread and pastas, which are vulnerable to β-glucanase activity, had low β-glucan solubility compared to the original unprocessed oats used to make the foods. The MW of both bread and pasta were lowered by β-glucanase activity during the preparation of these foods, which should have increased β-glucan solubility. However, as was the situation with oat bran muffins, polymer aggregation
in the absence of water yielded low β-glucan solubility from these foods. The findings from both studies exemplify how it is necessary to find a balance between β-glucan MW and solubility to obtain the most biologically active form of β-glucan.

1.5 PHYSIOLOGICAL PROPERTIES OF β-GLUCAN

The relationship between β-glucan structure and rheological behaviour gives rise to the physiological effects of β-glucan in humans, which include lowering blood glucose levels after meals (postprandial glycemic response), and reducing low-density lipoprotein (LDL) cholesterol levels for improved health.

1.5.1. β-glucan and Glycemic Response

1.5.1.1. Background Information

The postprandial glycemic response is normally characterized by a rise in blood glucose levels after the ingestion of a meal, followed by an increase in blood insulin levels. Insulin released from the pancreas is required for glucose to be taken up by the cells and for normal blood glucose levels to be restored. In Type 2 Diabetes (T2D), the body does not respond to insulin, leading to sustained high blood glucose levels or hyperglycemia after food consumption. If left untreated, this condition may lead to dire health consequences, such as blindness, coma, or even death. However, reducing postprandial glycemic response is associated with a decreased risk of developing T2D (4, 48-51), and is also important in the management of diabetes.

The addition of β-glucan to carbohydrate-containing foods has been shown to lower its glycemic index value (GI), which is a measure of the food’s potential to lower blood glucose levels compared to a control food. Jenkins et al. (8) tested the effect of several food
products containing different amounts of β-glucan on glycemic response, and found the GI of foods to decrease with increasing β-glucan content. Upon examination of the results from similar studies, it was calculated that an average 3.8-unit reduction in a food’s GI value can be achieved for every 1g β-glucan incorporated into the original food. Thus, the ability for β-glucan to attenuate the rise in blood glucose gives it potential to be used as a tool for diabetes prevention and control.

1.5.1.2. The Role of Viscosity and Related Factors on Glycemic Response

The main mechanisms with which β-glucan is thought to affect glycemic response are related to β-glucan solution viscosity (6, 14, 52). When β-glucan and available carbohydrates (absorbable carbohydrates) are consumed together, the soluble fibre increases the viscosity of the contents under digestion, trapping the available carbohydrates in the food and hindering digestive enzymes from releasing those carbohydrates from the food matrix. Furthermore, the viscosity of the solutions in the gut may delay gastric emptying of food into the small intestine, as well as act as a barrier to carbohydrate absorption by coating the intestinal surface and slowing the entry of available carbohydrates into the bloodstream. These activities together result in an overall attenuated glycemic response compared to the response elicited by a meal without β-glucan.

The effect of β-glucan on blood glucose levels has been demonstrated in both drinks (6, 40, 53-55) and solid foods (56-58). The incorporation of β-glucan into glucose solution has proven to be an effective drink model for studying β-glucan rheological properties on glycemic response. By altering the concentration (C) of β-glucan in glucose drinks and varying the MW of the polymers in solution, Wood et al. (6) produced β-glucan solutions which varied in viscosity and thus strength of glycemic response-lowering. The
establishment of the linear relationship between log (viscosity) of a \( \beta \)-glucan solution and the log (C x MW) of the polymers in solution has revealed the importance of C and MW in affecting \( \beta \)-glucan flow behaviour and thus bioactivity (7).

Since solution viscosity has been shown to be a key factor associated with lowering blood glucose levels, MW and C are therefore important determinants of glycemic response. By extension, factors which affect \( \beta \)-glucan MW and C have indirect effects on the degree to which blood glucose is lowered. For instance, the method used to extract \( \beta \)-glucan from the native grain may lower \( \beta \)-glucan MW, which translates to a weaker glycemic response lowering-effect. In a study by Panahi et al. (55) subjects were served glucose drinks containing \( \beta \)-glucan obtained from two different extraction methods, one of which yielded \( \beta \)-glucan with greater polymer degradation. Although both fibre drinks elicited lower glycemic responses and had higher viscosities than a control glucose drink, the drink with less depolymerisation achieved the greatest attenuation in glycemic response.

In foods containing \( \beta \)-glucan, the effect of processing is known to affect C and MW, and thereby affect glycemic response. Regand et al. (47) showed that the glycemic response elicited by oat food products enriched with \( \beta \)-glucan and by similar food products with low \( \beta \)-glucan content, varied with the type of processing applied to the foods. For example, the glycemic response elicited by oat crisp bread with 4g \( \beta \)-glucan was comparable to that of control wheat crisp bread containing only 1g \( \beta \)-glucan (47). The lack of dose response was due to the presence of \( \beta \)-glucanases in oat bran and wheat flour. The relatively long preparation time required to make bread dough allowed \( \beta \)-glucanases to extensively degrade \( \beta \)-glucan polymers, reducing both MW and strength of effect on glycemic response. In contrast, oat granola and oat porridge had the greatest response lowering-effects compared to
control wheat muffin. This was explained by the retention of high β-glucan MW due to relatively short food preparation times for granola and porridge, and thus a limited time frame for β-glucanase activity to occur (47).

The foods of this study were also placed under *in vitro* digestion and solubilized β-glucan was extracted for measurement of viscosity (47). β-glucan extracts obtained in this manner are representative of the β-glucan solutions which develop in the gut during digestion, and while they are not exact replicates of digested fluids, their properties may help elucidate how β-glucan acts *in vivo* in order to lower glycemic response. In the study by Regand *et al.* (47), the extracts obtained from foods with higher MW had higher viscosity values, and extract viscosity was negatively correlated with glycemic response. Interestingly, the extract from oat crisp contained the greatest amount of solubilized β-glucan and thus the highest C of β-glucan in solution, but one of the weakest effects of lowering glycemic response. β-glucanase activity again explains this finding, as the enzymes freed β-glucan from oat bran cell walls to increase the amount of soluble β-glucan but simultaneously decreased the MW of β-glucan polymers (47). Achieving high C and high MW in the food matrix is complex but necessary to optimize β-glucan bioactivity.

In another study (41), freeze-thaw cycling of oat bran muffins was shown to affect β-glucan C and alter extract viscosity. Although a lower MW should increase polymer solubility, extracts from muffins with low MW β-glucan had the lowest β-glucan solubility, and this phenomenon was similar to the ones explained for the studies by Tosh *et al.* (9) and Regand *et al.* (47) above (section 1.4). Freezing of the muffins caused polymers to concentrate and form insoluble aggregates, while thawing mobilized more polymers and led to additional aggregate formation. As is true in β-glucan gel formation, low MW polymers
are less stearically hindered from associating with one another, and thus, aggregate formation would have been greater in low MW muffins. Accordingly, low β-glucan solubility meant low extract viscosity, which manifested as a weak lowering effect on glycemic response in vivo. Therefore, these studies in foods demonstrate how β-glucan solution viscosity and therefore bioactivity can be altered at many stages before the foods are consumed, which may explain the presence or lack of effect on glycemic response.

Further illustrating this fact is the study by Poppit et al. (59) which measured glycemic response in subjects served glucose drinks with or without the addition of barley β-glucan. In this study, no differences in blood glucose concentration between control and treatment groups were found. However, the fibre-containing drink was described as having a “clearly visible particle suspension which, when consumed, had the texture of pithy orange juice(59).” This suggests that the fibre was not properly solubilized in the drink treatment. If β-glucan is not solubilized in solution, viscosity does not develop, which could explain the lack of effect on glycemic response. Also, since the MW of β-glucan used was not reported, it is possible that the MW was too low to cause a reduction in glycemic response.

Preparation and processing are important to consider for the resulting effect on glycemic response, but the actual environment in which β-glucan is incorporated is another factor requiring attention. In the study by Regand et al. (47), oat and wheat pasta elicited the lowest glycemic responses of all foods tested, despite that the β-glucan MW of both foods were not the highest. This anomaly is explained by the inherent ability for pastas to elicit a low glycemic response, which is presumably due to its high density food form as a result of being produced by extrusion. The typical compact structure of pasta allowed for the following to occur in this study: firstly, for the wheat pasta to be digested slowly and to elicit
a glycemic response similar to that of oat pasta; and secondly, for the oat pasta to elicit a lower glycemic response than other oat foods that had higher extract viscosity. In the study by Tosh et al. (9) mentioned above (section 1.4), β-glucan dose and MW were varied in oat bran muffins, but an increase in β-glucan MW and C was not necessarily followed by a stronger lowering effect on glycemic response. When MW and dose were increased, glycemic response lowering increased initially but then decreased at higher MW. The limited amount of water in the food environment made it difficult for β-glucan at high dose and high MW to solubilize. Therefore, a lower solubility in β-glucan resulted in a low extract viscosity and thus a weaker effect on glycemic response.

The deviation from the established relationship between viscosity, MW, and C that was observed in the study by Tosh et al. (9) demonstrates the utility of testing β-glucan on glycemic response in the form of liquid solutions. Administering glucose solutions with and without β-glucan fibre eliminates any confounding effects of the food matrix on the ability of the polysaccharide to lower blood glucose levels. Moreover, whereas extract viscosity from foods must be obtained through in vitro digestion, the viscosity of a β-glucan solution itself is representative of the solution viscosity in the gut (5). For these reasons, it remains important to use β-glucan drink models to investigate the rheological properties of β-glucan and how they relate to glycemic response.

1.5.1.3. Additional Mechanisms for β-glucan Glycemic Response – Lowering

A mechanism unrelated to viscosity development has also been proposed for how β-glucan may lower glycemic response. There is evidence that the affinity of β-glucan for water may affect the rate of starch digestion in foods, which in turn affects the rate at which glucose enters the bloodstream. Starch gelatinization occurs when it is hydrolysed with
water, making it susceptible to amylase degradation during digestion (60). In β-glucan containing-foods, competition for water in the food matrix takes place between β-glucan and starch, and the amount of water absorbed by each may depend on the proportion of β-glucan versus starch in the food. This affects the amount of starch that is digestible, as well as how easily the starch is digested.

Regand et al. (61) showed that granola containing 40g or 60g starch in addition to 5g β-glucan had a higher ratio of slowly digestible starch (SDS) to rapidly digestible starch (RDS) than the same granola mixtures without β-glucan. The results suggest that higher β-glucan content decreases the amount of water available for starch gelatinization, leading to a greater proportion of SDS, which is the type of starch that is less readily digested. When the granola mixtures containing β-glucan and 40g or 60g starch were compared to each other, RDS was higher in 60g-starch mixture, which is explained by the lower proportion of β-glucan in that mixture and thus more water available for starch gelatinization. Furthermore, significant correlations were seen between increasing glycemic response (represented by AUC) and amount of RDS for β-glucan mixtures with 40g and 60g starch (61). There were also inverse correlations between glycemic response and SDS, but this was only significant for the β-glucan mixture containing 40g starch. Nevertheless, this study effectively demonstrated a plausible relationship between the rate of starch digestion, the presence and amount of β-glucan, and the effect on glycemic response. In other words, β-glucan slows the degradation of starch which then leads to a reduced rate of glucose release into the blood. More studies are needed to investigate the degree to which this mechanism is involved in how β-glucan affects glycemic response.
Others have suggested that the metabolic effects exerted by the products of colonic fermentation on β-glucan may contribute to the glycemic response lowering effect. Unlike the enzymes of the human digestive tract, colonic bacteria are able to break down β-glucan fibre as an energy source. The fermentation process produces short-chain fatty acids as by-products, including acetate and propionate, which were once thought to affect blood glucose levels by affecting either insulin or glucagon secretion. Although it has been shown that changes in glucagon and insulin levels are not significantly affected by either of the fatty-acids (62), a study by Acheson et al. (63) was conducted to test whether β-glucan lowers glycemic response through mechanisms other than a delayed absorption of carbohydrates. Isocaloric diets with or without 8.9g β-glucan/day were administered as 9 consecutive hourly meals. It was predicted that the partitioning of β-glucan consumption over several hours would prevent sufficient solution viscosity from forming in the gut, thus eliminating the effect of delayed glucose absorption by β-glucan. If differences in glycemic response between the two diets were observed, then β-glucan was acting on blood glucose levels through a mechanism other than viscosity development. However, differences were not detected. Furthermore, radioactive carbon tracers revealed a reduction in exogenous glucose production at the 7th meal in the systemic circulation of subjects given the β-glucan diet, meaning that a delay in carbohydrate absorption had occurred due to the cumulative effects of previous β-glucan doses given earlier in the feeding regimen. Meanwhile, endogenous glucose production was similar between the two groups. The results indicated that glycemic response lowering by β-glucan is not mediated through metabolic effects which alter endogenous glucose production (63). This study thus lends further support for the mechanism of β-glucan solution viscosity on glycemic response lowering. Moreover, the hypothesis for
delayed starch digestion due to the presence of β-glucan is left entirely plausible. It appears likely that both viscosity and starch digestion are important in the delayed release and absorption of available carbohydrates in the circulation. In fact, Behall et al. (64) showed that muffins containing both resistant starch (starch that remains undigested and appears in the colon) and β-glucan elicited a greater glycemic response lowering-effect than muffins containing either β-glucan or resistant starch alone.

1.5.1.4. Short-Term versus Long-Term Effects of β-glucan on Glycemic Response

In general, the consumption of β-glucan-rich foods has been shown to assist both healthy subjects and Type 2 diabetic (T2D) subjects in short-term glycemic control. The long-term effects, however, are less clear. Cugnet-Anceau et al. (65) found no differences in levels of fasting blood glucose or glycated haemoglobin between T2D control subjects and T2D subjects who consumed 3.5g β-glucan daily for 8 weeks. Similarly, Biorklund et al. (66) found no difference in fasting blood glucose levels of normoglycemic subjects before and after a 5-week period during which subjects consumed β-glucan rich beverages daily. In the study by Frank et al. (67), the long-term effect of consuming high MW β-glucan rich bread for 3 weeks was compared to that of consuming low MW β-glucan bread for 3 weeks, but no differences in fasting blood glucose were found between the groups at the end of the intervention period. In contrast, Garg et al. (68) showed that T2D males consuming β-glucan enriched oat bran bread for 12 weeks were able to achieve a lower glycemic response than after consuming white bread for 12 weeks. Thus, perhaps the duration of the intervention periods in the former three studies were insufficient for an effect on glycemic control to be observed. Furthermore, since the MW used in the study by Cugnet-Anceau et al. (65) was only 80,000g/mol, while the MW in Biorklund et al.’s study (66) was even less at
70,000 g/mol and 40,000 g/mol for the high and low MW β-glucans respectively, MW may have been too low to have long-term effects on glycemic response. It thus appears that consumption of β-glucans for periods longer than 8 weeks may be necessary to affect long-term glycemic control, and the properties of the β-glucan consumed, in particular MW, remains important. In addition, although these studies did not show long-term effects on blood glucose lowering, the short-term effect of lowering postprandial glycemic response was observed, which still lends support for β-glucan to be used as a therapeutic tool in achieving glycemic control.

1.5.2. Other Health Benefits of β-glucan

Another health benefit for which β-glucan has garnered much attention is its ability to lower blood cholesterol levels, leading to a reduced risk of cardiovascular disease. After critically evaluating past and current scientific evidence, Health Canada, the United States Food and Drug Administration, and the European Food Safety Authority have all approved health claims recognizing that 3g/day of oat β-glucan may reduce serum cholesterol levels (69-73). Despite the large body of evidence supporting the claims, evidence against the claims raises legitimate concerns as to the conditions, such as dose and vehicle of administration, that are required for oat β-glucan to effectively lower cholesterol levels.

1.5.2.1. β-glucan and Cholesterol-Lowering

It is believed that the viscous β-glucan solutions which form in the gut line the intestinal surface, preventing both the absorption of cholesterol and re-absorption of bile acids during digestion. The body then compensates for the loss of bile acids by removing cholesterol from the blood to synthesize new bile acids (72). As a result, blood cholesterol levels are reduced.
Similar to how β-glucan affects glycemic response, viscosity may play a role in how β-glucan lowers cholesterol levels, which means that MW and C are also important. In a study by Wolever et al. (74), ready-to-eat wheat cereal or oat bran cereal were administered to healthy subjects over 4 weeks. The oat bran cereals were specifically manufactured to provide either 4g low MW (4L), 3g medium MW (3M), 4 g medium MW (4M), or 3 g high MW (3H) β-glucan per day. By varying MW and dose, the viscosity of the β-glucan solutions in vivo were presumed to differ. At 4 weeks, subjects receiving either 3H or 4M cereal had significantly lower levels of low-density lipoprotein (LDL) cholesterol than those who consumed wheat cereal, while LDL levels for 3M and 3L cereals were similar to that of wheat cereal. Since medium and high MW cereals differed from the control, the results suggest that higher MW strengthens cholesterol-lowering. In addition, since the 4M but not 3M cereal differed from control, the study demonstrated that higher dose also leads to lower cholesterol levels. High MW and dose is related to β-glucan solution viscosity, and so the viscosity of β-glucan extracts obtained from in vitro digestion of the cereals was measured. The study found a significant inverse relationship between LDL levels at 4 weeks and log (viscosity), as well as with log (C x MW) of the extracts (74). This showed that viscosity, and thus C and MW, may indeed play a role in cholesterol-lowering.

The finding that β-glucan C and MW are important for lowering cholesterol provides an explanation as to why some studies (75, 76) but not others show positive results for β-glucan. For instance, Biorklund et al. (66) tested the effect of oat and barley β-glucan incorporated in drinks on LDL levels and found no significant differences between barley β-glucan drinks and control rice drink. However, the MW of the barley β-glucan was only 40,000g/mol which may have been insufficient to effectively lower LDL levels.
As discussed, β-glucan MW may be altered during food processing and storage, and thus the food matrix is also an important aspect to consider for β-glucan bioactivity. In two separate experiments, Kerckhoffs et al. (77) investigated the role of food form in the effect of β-glucan on cholesterol levels. In the first experiment, subjects consumed for 4 weeks either cookies and bread which provided a total of about 5g β-glucan per day, or control cookies and bread containing no β-glucan. The consumption of 5 slices of bread daily was required whereas the cookies were consumed ad libitum, although it was reported that only about 13% of β-glucan consumed per day was from cookies. The results showed no differences in LDL levels between control and treatment groups (77). However, it was noted that the MW of bread was heavily reduced due to β-glucanase activity during bread-making, while >80% of β-glucan in cookies was greater than 1,000,000 g/mol. Since most of the β-glucan consumed was from the oat bread of compromised MW, subjects may not have received β-glucan of sufficient MW to achieve the lower LDL levels desired. Interestingly, the study reported that in the preparation of oat bread, oat bran was soaked in water to help leaven the bread, and that a “light gel” had formed during this process. The effect of β-glucan gels on both glycemic response and blood lipid levels are unknown.

In the second experiment (77), subjects consumed orange juice with or without β-glucan, and LDL cholesterol was significantly lower in the treatment group compared to control. Overall, the study shows that the effectiveness of β-glucan may be altered by food processing and may depend on the food matrix in which the β-glucan is incorporated. Furthermore, it is possible that administering β-glucan in liquid form rather than solid foods is more effective for lowering LDL cholesterol. The findings from Naumann et al. (75) support this idea, as fruit juice containing 5g oat β-glucan yielded significantly lower LDL
levels than control fruit drink after daily consumption for 5 weeks. In contrast, the study by Cugnet-Anceau et al. (65) showed no changes in LDL or total cholesterol between subjects consuming β-glucan enriched soups and subjects consuming soups without β-glucan. However, the β-glucan MW in this study was 80,000 g/mol, which may have been too low for significant changes in LDL levels to be exhibited. In general, there is more evidence in support of, rather than against, the lowering effect of β-glucan on LDL cholesterol, but just as with glycemic response studies, factors such as MW, C, and food matrix must be carefully considered in order to achieve accurate results regarding the health effects of β-glucan.

1.6 THE EFFECT OF LIQUID VOLUME ON GLYCEMIC RESPONSE

Food volume has been shown to affect the rate of gastric emptying, which in turn affects postprandial glycemic response. However, the results of studies that have investigated the effect of volume on blood sugar levels have been contradictory.

Sievenpiper et al. (78-80) conducted several studies to determine the effect of volume on glycemic response. Their first study showed that increasing the volume of a 25g oral glucose solution from 200ml to 600ml increases the AUC in healthy subjects, and that the blood glucose concentration was higher at 15 minutes into the blood sampling period after consuming the 600ml beverage (78). In line with these results, their second study which was also conducted in healthy subjects showed that a 75g glucose solution at 900ml resulted in a greater AUC and higher peak glucose value than at 600ml or 300ml (79). These findings point towards a trend of increasing glycemic response with increasing volume. However, their third study showed that a 300ml solution elicited a greater glycemic response than 600ml or 900ml volumes in lean and normal subjects, while there was no effect of volume in obese subjects (80). The reason for the discrepancies between the three studies is unknown,
but the results of the third study suggest differential effects of volume on glycemic response depending on the characteristics of the subject group, which could include BMI and glucose tolerance. Age, which is often a determinant of health, may also be a factor, since a study conducted in older adults (mean age 73.9 ± 1.2 years) (81) found that tripling the volume of either a 200ml, 25g or 200ml, 75g oral glucose solution had no effect on glycemic response. Thus, at least some of the reported differences in the effects of volume are likely related to differences in the age and health status of subject groups, making it difficult to compare results across studies.

Torsdottir et al. (82) also found volume effects to differ in different types of subjects. They found that adding 300ml water to a mixed meal of meat and potatoes increased the AUC. The same results were found in T2D subjects with good glycemic control (HbA1c < 8.0%), but there was no effect of diluting the meal in T2D subjects with poor glycemic control (HbA1c > 8.0%). Gregerrson et al. (83), on the other hand, found no difference in AUC of T2D subjects when a meal of rye bread, tomatoes and butter were served with either 90ml or 600ml water. However, the lack of effect may be due to the low glycemic nature of the meal, which could prevent any significant difference of the added water volume from being observed. The results of this study are a reminder that the composition of the test meal is important to consider when glycemic response testing is involved.

Besides the effect on AUC, volume has also been shown to affect the shape of the glycemic response curve. A study by Young et al. (84) found no effect of volume on AUC, but did detect an effect on the pattern of the glycemic response curves. Subjects were given test meal bars containing 50g available carbohydrates with water at volumes of 50ml, 250ml, 500ml, 750ml, and 1000ml. The peak rise in blood glucose was the highest and reached the
earliest for the 500ml and 750ml solutions. However, the 500ml and 750ml solutions also had the lowest blood glucose concentrations at the end of the 2-hour sampling period. Interestingly, the effect of 1000ml on glycemic response was comparable to that of 250ml. It would appear that small volumes such as 50ml result in a lower peak rise and more gradual glycemic response compared to the larger volumes of 500ml and 750ml, which give a higher peak rise and more rapid fall in blood glucose levels. However, at larger volumes such as 1000ml, this trend is lost, suggesting a nonlinear effect of volume on the shape of the glycemic response curve (84). Since it is unclear whether volume plays a role in affecting the final glycemic response, it is important to include control foods with the same volume as the foods being tested.

In general, there is a paucity of research on how volume affects blood glucose levels, and more studies regarding this issue should be conducted.
1.7 RATIONALE FOR THE PROPOSED STUDY

β-glucan bioactivity depends on its rheological properties, which in turn depends on its physical properties. Thus, to achieve the desirable attenuation of glycemic response, it is important to know how varying these β-glucan properties may enhance or destroy the beneficial effect that this fibre has on health. Despite the research that has already been conducted with regards to the complex relationship between β-glucan physical properties, rheological properties, and bioactivity, there are questions which remain unanswered.

Solution viscosity may be maximized by increasing MW and C or dose of β-glucan when solution volume is constant in order to achieve a lower glycemic response. However, the effect on glycemic response when the solution volume is varied and β-glucan dose and MW are constant has not been investigated. Also, β-glucan has been shown to successfully lower glycemic response in liquid and solid food forms, but its effects when administered as a β-glucan hard gel have not been tested. Thus, this study aims to address the following questions:

1) How does β-glucan affect glycemic response when solution viscosity is altered through changes in solution volume while β-glucan dose remains constant?

2) Are β-glucan gels effective in lowering glycemic response?

The ability for oat β-glucan to confer significant health benefits has been discussed, and the viability of using β-glucan as a functional food ingredient has been shown, both as a natural part of the native cereal grain and as an extract incorporated into foods. Considering the mass potential for β-glucan to be utilized as a preventative tool and possible treatment for disease, conducting studies on the relationship between β-glucan function and structure is warranted.
CHAPTER 2

MATERIALS AND METHODS
2.1 STUDY DESIGN

This study followed a randomized block design with repeated measures. The study consisted of two independent experiments (Experiments 1 and 2) conducted simultaneously. Healthy subjects visited the study site on 10 separate occasions to participate in a series of 10 glycemic response tests. Each subject consumed 6 different test foods from Experiment 1, and 4 different test foods from Experiment 2, for a total of 10 test foods for the entire study. Foods were tested in random order, and each subject received each food once during the study. The physiochemical properties of the foods were measured to determine whether these properties relate to effects on glycemic response. In addition, glucose diffusion from the foods of Experiment 2 was analyzed in vitro. The methods for this in vitro experiment are discussed in section 2.7 below.

2.2 SUBJECTS

Fifteen healthy subjects (7 males, 8 females) were recruited for this study. Subjects were excluded if they were under 18 or over 75 years of age, had a body mass index (BMI) equal to or above 35, or were known to have diabetes, HIV, AIDS, hepatitis or a heart condition. Exclusion criteria also included the use of medications or having a condition which may harm the subjects or affect the study results. Subject characteristics are summarized in Table 2.1. This study was approved by the Health Sciences Research Ethics Board of the University of Toronto. Written informed consent was obtained from all subjects, and participants received financial compensation for completing the study.
### Table 2.1  Subject characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>37.2 ± 11.1</td>
<td>24.0 – 53.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.7 ± 18.7</td>
<td>50.4 – 111.8</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 ± 0.122</td>
<td>1.51 – 1.91</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 ± 4.10</td>
<td>21.4 – 34.6</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=15)

### 2.3  STUDY PROTOCOL

2.3.1.  **Glycemic Response Testing**

Participants visited the study site following a 10-12 hour overnight fast. Upon arrival to the study site, the height and weight of the subjects were measured. Two fasting blood samples spaced 5 minutes apart (-5 min and 0 min) were collected by finger-prick using a monoejector lancet device. Immediately following the collection of the second blood sample, subjects consumed a test food and 250mL of a beverage of their choice (water, tea or coffee with milk and/or artificial sweetener aspartame). Subjects received the same choice of beverage and volume of that beverage for each of the 10 tests in the study. Additional finger-prick blood samples were taken at 10, 20, 30, 40, 50, 60, 90 and 120 minutes after the start of the meal. Blood samples (2-3 drops each sample) were collected in fluoro-oxalate tubes to prevent coagulation, immediately stored at -20°C, and analyzed within 72 hours. Subjects remained seated for the duration of the test. Glucose concentration was analyzed using an automatic analyzer (Yellow Spring Instruments, 2300 Stat). The peak rise in blood glucose
concentration and the incremental area under the glycemic response curve (AUC) elicited by each food was determined according to the methods by Wolever et al. (85).

2.3.2. Choice of Blood Sampling Schedule

In glycemic response testing, blood samples are normally taken at 15-minute intervals (15, 30, 45, 60, 90 and 120 minutes) after the test food is consumed. However, blood was collected at 10-minute intervals in this study because there is evidence that sampling at every 10 minutes (which allows for more data points of blood glucose concentration,) may yield a more accurate measurement of peak rise. A study by Lui et al. (86) showed that sampling at 10-minute intervals resulted in peak rise values being 4% greater than values measured from sampling at 15-minute intervals. Furthermore, sampling at every 10 minutes resulted in greater differences in peak rise values between foods being tested, meaning that the peak rise was more distinguishable between test foods when sampling was done at 10-minute intervals. Peak rise values may be more important than AUC when describing the glycemic response elicited by viscous soluble fibres such as β-glucan (9, 47). This is due to the mechanism with which β-glucan attenuates glycemic response, which involves suppressing the maximum glucose concentration attained while prolonging the fall in blood glucose back to fasting level. This makes the β-glucan curve flatter and broader than that of the control, which means β-glucan tends to lower the peak rise compared to the control food, but may retain a similar AUC as the control. Since peak rise may better describe how β-glucan affects glycemic response, sampling for this study was done at 10-minute intervals in order to achieve greater accuracy in measuring peak rise and differentiating peak rise between foods.
2.4 TEST FOODS

2.4.1. **Description**

Test foods for Experiments 1 and 2 were provided by the Guelph Food Research Centre and Megazyme International. Each food was either a solid hard gel or a solution containing 50g of anhydrous dextrose prepared with or without the addition of 4g purified oat β-glucan fibre (Megazyme International). The 50g glucose load acted as the available carbohydrate in the meal that is needed to elicit a glycemic response. The β-glucan fibre was extracted from oat bran and purified to remove starch, proteins and arabinoxylan. The desired molecular weight levels were achieved by enzymatic hydrolysis of β-glucan with lichenase. **Table 2.2** shows the composition of each food. Drinks are named to correspond to their volume (130mL, 250mL or 600mL) and to indicate the presence or absence of β-glucan fibre. Drinks containing no β-glucan are designated with the letter ‘N’. Drinks containing β-glucan are designated with the letters ‘H’ or ‘L’ which describe the relative molecular weights (MW) of β-glucan used in this specific study (‘H’ for high or ‘L’ for low). Thus, 130N is a drink that is 130mL in volume and contains no β-glucan, while 250H is a drink that is 250mL in volume and contains 4g high MW β-glucan. The hard gel which contains low MW β-glucan has been named LMWG. Test foods were analyzed for their rheological properties in a food analysis lab.

In order to prevent bacterial and mould growth in the drinks and gel, 0.05% potassium sorbate solution was added to the foods as a preservative. The 0.05% potassium sorbate solution was prepared by dissolving 2g potassium sorbate powder in 4L of distilled water at room temperature.
2.4.2. Rationale for the Specific Use of Oat β-glucan

The molecular structure, physical properties, and rheological properties of oat β-glucan are well-characterized (14). β-glucan can be found in several cereal grains not limited to oats. In fact, β-glucan from barley and rye possess a higher DP3:DP4 ratio and form faster gels than oat β-glucan (2, 23). However, international food regulatory bodies (United States Food and Drug Administration, European Food Safety Authority, Health Canada) have recognized the health benefits of oat β-glucan, notably the effect the fibre has on lowering blood glucose and blood lipid levels. Furthermore, health claims in support of oat consumption to achieve these benefits are already in place (69, 70, 71). It is thus appropriate to use oat β-glucan in this study if functional foods are to be made with fibre from this cereal source. Furthermore, as oat β-glucan forms weaker gel networks than β-glucan from other sources (23), a positive effect of the hard gel in this experiment on lowering glycemic response would suggest a positive effect for β-glucan hard gels in which the gel network is stronger.

2.4.3. Drink Preparation

In a 1-L cylindrical Ziplock© container with a screw-on cap, dry ingredients were weighed to the amounts corresponding to the individual composition of each drink (Table 2.2). Treatment drinks contained β-glucan fibre in addition to dextrose, while control drinks contained no β-glucan fibre. The container was then sealed by screwing its cap tightly in place and gently shaken to mix the contents and to ensure that the β-glucan fibre was well-dispersed among the dextrose granules.

The cap was then removed and boiling 0.05% potassium sorbate solution was added to the dry ingredients at the volume which corresponded to the composition of the food.
Immediately after the addition of liquid, the solution was homogenized with a handheld blender until no particles of dry ingredients remained. Pure orange extract was added to the solution, followed by red and yellow food colouring to give the solution an orange flavour and colour respectively. The solution was further homogenized until smooth and no clumps were present. The drink was left to cool and to sit overnight (between 19 and 22 hours) at room temperature before being served the following day.

2.4.4. Gel Preparation

In a 250-ml cylindrical Ziplock© container with a screw-on cap, the wet and dry ingredients were measured, combined, and homogenized according to the methods described above for preparing drinks. However, the solution that was prepared for making LMWG was left to sit at room temperature for 7 days to allow the β-glucan gel to form. Thus, the β-glucan gel was served one week after being prepared, while β-glucan drinks were served one day after being prepared.
Table 2.2  Composition of test foods

<table>
<thead>
<tr>
<th></th>
<th>Test Food</th>
<th>Dextrose</th>
<th>High MW (\beta)-Glucan</th>
<th>Low MW (\beta)-Glucan</th>
<th>Citric Acid</th>
<th>Distilled Water with 0.05% Potassium Sorbate (mL)</th>
<th>Orange Extract (mL)</th>
<th>Red Food Colouring (mL)</th>
<th>Yellow Food Colouring (mL)</th>
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</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>250N</td>
<td></td>
<td>50.0</td>
<td>0</td>
<td>0</td>
<td>0.1</td>
<td>220</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>0.3</td>
<td>570</td>
<td>1.0</td>
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<td>4</td>
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<tr>
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<td>0.16</td>
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<tr>
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<td>0.41</td>
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<tr>
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<td>4.0</td>
<td>0.22</td>
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<tr>
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<td>0.44</td>
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<tr>
<td><strong>Experiment 2</strong></td>
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<td></td>
<td></td>
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<tr>
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<tr>
<td>130L</td>
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<tr>
<td>LMWG</td>
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<td>0.09</td>
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<td>0.25</td>
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</tr>
</tbody>
</table>
2.5 PHYSIOCHEMICAL PROPERTIES OF TEST FOODS

2.5.1. Molecular Weight Determination

β-glucan molecular weight (MW) was determined by high-performance size-exclusion chromatography (HPSEC) using the methods described in the study by Ragaee et al. (43) but with modifications. Briefly, the HPSEC system consisted of a Shodex OHpak KB-806M and Ultrahydrogel linear column with a Shimadzu SCL-10Avp pump and auto injector (Shimadzu Scientific Instruments, Columbia, MD). Elution was with 0.1M NaNO3 containing 0.03% (w/w) NaN3 at a flow rate of 0.6 mL/min. Chromatographic peaks were detected with a Viscotek TDA 305 detector which consists of a refractive index detector, a low angle laser light scattering detector, a right angle laser light scattering detector and a differential pressure detector. The Viscotek DM 400 data manager and OmniSEC software were used to determine the MW distributions of β-glucan standards and β-glucan used in test foods. Pullulan (J.M. Science, New York, NY) was used to calibrate the detectors. A refractive index increment (dn/dc) of 0.146 mL/g was used (33, 44). Values measured were weight average MW (g/mol).

2.5.2. Rheological Measurements

Apparent viscosity values for test foods from Experiment 1 were measured using the methods described in the study by Tosh et al. (9). Using a controlled strain rheometer (TA Instrument, ARES, New Castle, DE) fitted with a cone and plate geometry (cone angle of 0.04 radians, 50 mm diameter), apparent viscosity was measured in a shear rate range of 0.1 – 100s⁻¹ at 37°C. For statistical analysis, viscosities measured at the shear rate 32 s⁻¹ were used.
Viscoelasticity values for test foods of Experiment 2 were measured using the same controlled strain rheometer described above but under different conditions. For the drinks (130N, 130L and 130H) the conditions were as follows: cone angle of 0.05 radians, 50mm diameter, 0.05 gap. For the hard gel (LMWG), conditions were as follows: 25mm plate, 1 mm gap. Measurements were taken over a frequency range of 0.1-63s\(^{-1}\) under 2% strain at 37°C.

2.6 STATISTICAL ANALYSIS OF GLYCEMIC RESPONSE RESULTS

Peak rise, AUC, and the blood glucose concentrations at each time point were subjected to repeated measures of analysis of variance (ANOVA). After demonstration of significant heterogeneity, the significance of difference among individual measures was determined using Tukey’s Test. Additionally, for Experiment 1, linear regression analysis was used to determine the interaction between the physiochemical properties of the foods, as well as between those properties and the glycemic response elicited by the foods. A two-tailed p-value of <0.05 was taken as the criterion for statistical significance. Results were presented as mean ± SEM. All analyses were performed using Microsoft Excel Data Analysis Tool Pack. The number of subjects in this study allowed for differences between means to be detected with 80% power at a p-value of 0.05 for statistical significance.
2.7 *In vitro* GLUCOSE DIFFUSION ANALYSIS

2.7.1. Overview

Glucose diffusion analysis was performed on the β-glucan solutions and hard gel of Experiment 2 according to the methods by Jenkins *et al.* (87), cited by Lecumberri *et al.* (88), with modifications. Samples of each food were placed in water baths and glucose was allowed to diffuse from the foods into the surrounding water over a period of 2 hours. The glucose concentration of the water bath was measured at every 15 minutes to determine the amount of total glucose diffused from the food at the given time point. The amount of glucose diffused was expressed as a percentage of the starting amount of glucose in the food at 0 minutes.

2.7.2. Protocol

The amount of glucose diffused from the test foods was determined *in vitro* by placing samples of each food in dialysis tubing and incubating the tubes in heated water baths. Dialysis tubes were soaked in deionized water for 30 minutes in order to hydrate the tubes and make them pliable. Tubes were also washed with deionized water to rid of any glycerol adhered to the tubes. Samples of approximately 3.5g were taken from each test food and were weighed. The samples were placed in separate dialysis tubes. Each tube was incubated for 2 hours in its own water bath containing 500mL deionised water at 37°C and a rotating magnetic stir bar. Over the 2-hour period, glucose from each sample diffused out of the dialysis tube and into the surrounding water. At 15-minute intervals, 0.5mL-samples of water were transferred from the water bath into a clean glass tube. An additional 0.5mL of fresh deionised water (not from the water bath) was added to each tube before being analysed for glucose concentration.
A diagram of the experimental setup is shown below (Figure 2.1). Two full-sized samples of each food were prepared on two separate days. For 130N, 130L and 130H, glucose diffusion analysis was performed the same day that the foods were prepared. For LMWG, glucose diffusion analysis was performed 7 days after the food was prepared to allow sufficient time for gel formation. Two samples were taken from each full-sized food and placed in separate water baths for glucose diffusion analysis. From each water bath, two water samples were taken at each time point for measurement of glucose. This resulted in a total of 8 water samples per time point per food to be analysed for glucose. The glucose concentration of the water samples was determined using a glucose oxidase/peroxidase (GOPOD) assay kit (Megazyme International).
Figure 2.1 Setup of glucose diffusion analysis of a test food

Add 0.5mL deionised water to each sample

GOPOD assay for determination of glucose concentration

**Figure 2.1** Setup of glucose diffusion analysis of a test food
2.7.3. **Measurement of Glucose Concentration Using GOPOD**

The amount of glucose measured in the water samples taken at each time point was used to calculate the amount of glucose diffused from the food into the water bath at the corresponding time point. Glucose was determined using the procedure outlined in the manual of the Megazyme D-Glucose Asssay Kit (GOPOD-Format) (Megazyme International), with the exception that each 0.5mL sample of water removed from the water bath was diluted with 0.5mL deionised water prior to adding any reagents from the assay kit. The purpose of this was to dilute the samples because the glucose concentration of each sample was beyond the range of detection of the assay. Adding 0.5mL deionised water to the samples doubled the sample volume, thereby diluting the samples by a factor of two. Thus, the amount of glucose determined for each sample was half the true amount of glucose in the sample prior to dilution. The dilution was later corrected for by multiplying the value for glucose in the diluted sample by two.

As described in the manual, 0.1mL water from each sample (after the additional dilution step) was used for measurement of glucose. 3.0mL GOPOD reagent was added to each 0.1mL sample, as well as to 0.1mL of D-glucose standard (100ug/0.1mL) provided, and incubated at 40-50°C for 20 minutes. Absorbance was read at 510nm against the reagent blank. The amount of glucose determined in each sample was then multiplied by two to obtain the true amount of glucose in 0.1mL of the water bath. The amount of glucose in the entire water bath (500mL) could then be calculated.

\[
\text{Glucose (g) in water bath at a given time point} = 2 \times \left( \frac{\text{glucose determined (g)}}{0.1\text{mL sample}} \right) \times 5000
\]
2.7.4 Calculating the Percentage of Total Glucose Diffused

To determine the percentage of glucose that diffused from the dialysis bag, the total starting amount of glucose in the dialysis bag at 0 minutes of the experiment was calculated:

\[
\% \text{ weight of glucose in full-sized 130N} = \frac{\text{Weight of glucose}}{\text{Weight of full-sized 130N}} = \frac{50 \text{g glucose}}{50 \text{g glucose} + 100 \text{g water}} \times 100\% = 33.33\%
\]

\[
\% \text{ weight of glucose in full-sized 130H or 130L} = \frac{\text{Weight of glucose}}{\text{Weight of full-sized 130H or 130L}} = \frac{50 \text{g glucose}}{50 \text{g glucose} + 100 \text{g water} + 4 \text{g } \beta\text{-glucan}} \times 100\% = 32.47\%
\]

The starting amount of glucose in the dialysis bag was then obtained by multiplying its weight percentage in the full-sized test food by the weight of the test food sample in the dialysis bag.

The starting amount of glucose in the food sample was also the amount of glucose in the food sample at 0 minutes. Thus, the percentage of glucose that diffused from the food sample at a time point was determined by dividing the amount of glucose in water at a time point by the total starting amount of glucose in the dialysis bag, multiplied by 100%.

\[
\% \text{ Total Glucose Diffused} = \frac{\text{amount glucose in water}}{\text{amount total glucose in sample 0 minutes}} \times 100\%
\]
For each food, after the percentage of total glucose diffused at every time point was determined from 8 water samples, the 8 values were averaged to yield an average percentage total glucose released at the given time point. The average values were plotted to give curves of glucose diffusion from the foods. The average percentage total glucose released at 120 minutes in the analysis time frame was taken as the final average percentage total glucose released.

2.8 STATISTICAL ANALYSIS OF in vitro EXPERIMENT RESULTS

The final average percentage total glucose released was compared between the foods using analysis of variance (ANOVA). After demonstration of significant heterogeneity, the significance of difference among individual measures was determined using Tukey’s Test. Linear regression analysis was used to determine the interaction between the percent glucose released and the glycemic response elicited by the foods. A two-tailed p-value of <0.05 was taken as the criterion for statistical significance. Results were presented as mean ± SEM. All analyses were performed using Microsoft Excel Data Analysis Tool Pack.
CHAPTER 3

EXPERIMENT 1:

THE EFFECT OF CHANGING β-GLUCAN SOLUTION VISCOSITY BY ALTERING SOLUTION VOLUME ON GLYCEMIC RESPONSE
3.1 OBJECTIVE

To examine the effect of β-glucan solutions when solution viscosity is altered by changing the solution volume and holding the β-glucan dose constant.

3.2 MATERIALS AND METHODS

Refer to Chapter 2, Sections 2.1-2.6.

3.3 RESULTS

3.3.1. Effect of altering molecular weight (MW) and volume on solution concentration and viscosity

Solutions containing 4g β-glucan (250L, 600L, 250H, 600H) developed viscosity, whereas solutions without the addition of β-glucan (250N, 600N) achieved only a low viscosity level comparable to that of water. The viscosity of 250N and 600N may be attributed to the presence of glucose. Values for the dose of low and high MW β-glucan, solution volume, β-glucan concentration, β-glucan MW and apparent viscosity (simply referred to as viscosity herein) of the solutions are summarized in Table 3.1.

Table 3.1 Physiochemical characteristics of test foods for Experiment 1

<table>
<thead>
<tr>
<th>Food</th>
<th>β-glucan Dose (g)</th>
<th>Volume (L)</th>
<th>C (g/L)</th>
<th>MW (g/mol)</th>
<th>log(C x MW)</th>
<th>Viscosity ± SEM (mPa.s at 32s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600N</td>
<td>0</td>
<td>0.600</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1.03 ± 0.01</td>
</tr>
<tr>
<td>250N</td>
<td>0</td>
<td>0.250</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>1.48 ± 0.06</td>
</tr>
<tr>
<td>600L</td>
<td>4.00</td>
<td>0.600</td>
<td>6.70</td>
<td>1.45 x 10⁵</td>
<td>5.99</td>
<td>4.33 ± 0.04</td>
</tr>
<tr>
<td>250L</td>
<td>4.00</td>
<td>0.250</td>
<td>16.0</td>
<td>1.45 x 10⁵</td>
<td>6.37</td>
<td>39.1 ± 0.88</td>
</tr>
<tr>
<td>600H</td>
<td>4.00</td>
<td>0.600</td>
<td>6.70</td>
<td>5.80 x 10⁵</td>
<td>6.59</td>
<td>79.3 ± 2.18</td>
</tr>
<tr>
<td>250H</td>
<td>4.00</td>
<td>0.250</td>
<td>16.0</td>
<td>5.80 x 10⁵</td>
<td>6.97</td>
<td>2.05 x 10^1 ± 39.8</td>
</tr>
</tbody>
</table>

†Mean of viscosity values from three separate samples each measured three times at 32s⁻¹. SEM = standard error of the mean
Viscosity differed between solutions containing β-glucan fibre and having the same volume but different β-glucan MW (Figure 3.1). Thus, at the same β-glucan concentration, changing the MW changed the solution viscosity. Solutions containing high MW β-glucan (250H, 600H) had the highest viscosity, followed by solutions containing low MW β-glucan (250L, 600L). The glucose solutions containing no β-glucan (250N, 600N) were not viscous. Therefore, solution viscosity increased with increasing MW.

Viscosity also differed between solutions with β-glucan fibre and having the same β-glucan MW but different solution volume (Figure 3.1). At the same MW level, 250ml β-glucan solutions were more viscous than 600ml β-glucan solutions. Thus, increasing the volume lowered solution viscosity when β-glucan MW was the same. However, solution viscosity was the highest for solutions with higher β-glucan MW, regardless of volume.

![Figure 3.1](image)

**Figure 3.1** The effect of MW and volume on changes in log (viscosity) of β-glucan solutions at a constant β-glucan dose. (●) solutions with β-glucan; (○) solutions with glucose only.
A high correlation between \( \log(C \times MW) \) and \( \log(\text{viscosity}) \) was found \( (r^2=0.975, \ p<0.05) \), demonstrating the strong dependence of solution viscosity on \( \beta \)-glucan C and MW (Figure 3.2, Table 3.2). Data points for 250N and 600N were omitted because they did not contain \( \beta \)-glucan.

**Figure 3.2** The regression relationship between \( \log(C \times MW) \) and \( \log(\text{viscosity}) \)

![Graph showing the regression relationship between \( \log(C \times MW) \) and \( \log(\text{viscosity}) \)](image)

\[
y = -15.4 + 2.66x \\
\text{ }r^2 = 0.974
\]

**Table 3.2** The regression relationship between \( \log(C \times MW) \) and \( \log(\text{viscosity}) \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>a</th>
<th>SEE</th>
<th>b</th>
<th>SEE</th>
<th>( \text{r}^2 )</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>log (viscosity)</td>
<td>-15.4</td>
<td>1.98</td>
<td>2.66</td>
<td>0.305</td>
<td>0.974</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Regression equation is in the form: \( \text{variable} = a + b \times \log(C \times MW) \)

SEE = standard error of the estimate of ‘a’ and ‘b’

\( r^2 \) = correlation coefficient

* Significance of the regression relationship
3.3.2. Effect of altering viscosity on glycemic response

The glycemic response curves elicited by each solution over 120 minutes after fasting are shown below (Figure 3.3 (a) – (e)). Figure 3.3 (a) and (b) show the curves for solutions of 600ml volume and 250ml volume respectively, and illustrate the effect of β-glucan MW on glycemic response. Since there were three levels of MW for every level of volume, there are three curves (N, L, H) for each of Figures (a) and (b). At the same volume, a higher MW tended to yield a lower peak rise in blood glucose, as well as a broader and flatter shape to the curve. This is evident at both volumes as blood glucose concentration for 600H was significantly lower than that of 600N at 20 and 30 minutes (p<0.05), while blood glucose concentration for 250H was lower than that of 250N at 10 and 20 minutes (p<0.05). At the same volume, high MW solutions yielded the lowest blood glucose concentration at 120 minutes.

Figures 3.3 (c), (d) and (e) show the curves of the solutions containing no β-glucan, low MW β-glucan, and high MW β-glucan respectively, and illustrate the effect of β-glucan solution volume on glycemic response. Since there were two volume levels for each MW level, two curves (250 and 600) are shown in each figure. Figure (c) shows the effect of volume on glycemic response when only glucose is present. A higher volume tends to elicit a steeper and higher rise in blood glucose levels. Figure (d) shows the effect of volume when low MW β-glucan is added to glucose solution. At a higher volume, 600L gives a slightly higher maximum glucose concentration than 250L, but this effect was blunted by the addition of β-glucan. Figure (e) shows a similar effect of volume with high MW β-glucan present, where the maximum blood glucose reached is comparable between high and low volumes.
However, the higher volume of 600H still tended to give a steeper rise in blood glucose than 250H, with a significantly higher concentration at 10 minutes.
Figure 3.3 (a-e) Glycemic response curves elicited by foods of Experiment 1. The effects of MW on the blood glucose curve are shown at a volume of 600mL (a) and at a volume of 250mL (b). The effects of volume on the blood glucose curve are shown when no β-glucan is present (c); when low MW β-glucan is present (d); and when high MW β-glucan is present (e). Data points with different letters differ significantly (p<0.05). Values are mean ± SEM.
(c) **Glucose-only controls**

- 250N
- 600N

(d) **LMW**

- 250L
- 600L

(e) **HMW**

- 250H
- 600H
A simple linear regression model was applied to assess the correlation between log (viscosity) and mean peak rise (Figure 3.4), and between log (C x MW) and mean peak rise (Figure 3.5, Table 3.3). There was a significant trend of decreasing peak rise with increasing log (viscosity) ($r^2 = 0.759$, $p<0.05$). A trend of decreasing peak rise with increasing log (C x MW) was also observed, although this was not significant ($r^2 = 0.638$, $p>0.05$).

Correlations between log (viscosity) and mean incremental area under the glycemic response curve (AUC), as well as between log (C x MW) and mean AUC were also assessed. As shown in Figures 3.6 and 3.7 and Table 3.4, although trends of decreasing AUC with increasing log (viscosity) and decreasing log (C x MW) were present ($r^2 = 0.416$ and $r^2 = 0.278$ respectively) neither were significant ($p>0.05$).

Where regression analysis involved log (C x MW), data points for 250N and 600N were omitted because they did not contain β-glucan.
Regression equation is in the form: variable = a + bx; a, intercept; b, slope; x, independent variable
SEE = standard error of the estimate of ‘a’ and ‘b’
r^2 = correlation coefficient
* Significance of the regression relationship

Figure 3.4 The regression relationship between log (viscosity) and peak rise in blood glucose concentration.

Figure 3.5 The regression relationship between log (C x MW) and peak rise in blood glucose concentration.

Table 3.3 The regression relationship between log (viscosity) and peak rise, and between log (C x MW) and peak rise in blood glucose concentration.

<table>
<thead>
<tr>
<th>Variable</th>
<th>x</th>
<th>a</th>
<th>SEE</th>
<th>b</th>
<th>SEE</th>
<th>r^2</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Rise</td>
<td>log (viscosity)</td>
<td>4.12</td>
<td>0.160</td>
<td>-0.331</td>
<td>0.09</td>
<td>0.759</td>
<td>0.024</td>
</tr>
<tr>
<td>log (C x MW)</td>
<td></td>
<td>8.53</td>
<td>2.70</td>
<td>-0.781</td>
<td>0.416</td>
<td>0.638</td>
<td>0.201</td>
</tr>
</tbody>
</table>
Figure 3.6  The regression relationship between log (viscosity) and AUC.

![Graph showing regression line for log (viscosity) vs AUC with equation y = 2.37x10^2 - 9.54x and r^2 = 0.416.]

Figure 3.7  The regression relationship between log (C x MW) and AUC.

![Graph showing regression line for log (C x MW) vs AUC with equation y = 3.90x10^2 - 26.4x and r^2 = 0.278.]

Table 3.4  The regression relationship between log (viscosity) and AUC, and between log (C x MW) and AUC.

<table>
<thead>
<tr>
<th>Variable</th>
<th>x</th>
<th>a</th>
<th>SEE</th>
<th>b</th>
<th>SEE</th>
<th>r^2</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC</td>
<td>log (viscosity)</td>
<td>2.37x10^2</td>
<td>9.66</td>
<td>-9.54</td>
<td>5.65</td>
<td>0.416</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>log (C x MW)</td>
<td>3.90x10^2</td>
<td>1.95x10^2</td>
<td>-26.4</td>
<td>30.1</td>
<td>0.278</td>
<td>0.473</td>
</tr>
</tbody>
</table>

Regression equation is in the form: variable = a + bx; a, intercept; b, slope; x, independent variable
SEE = standard error of the estimate of ‘a’ and ‘b’
r^2 = correlation coefficient
* Significance of the regression relationship
Differences in peak rise between drinks were detected (p<0.0001) (Figure 3.8 (a)). Peak rise values for 250N, 600N, and 250L were significantly greater than those of 250H and 600H, while the peak rise for 600L did not differ from any of the other drinks. There were no significant differences in AUC between any of the drinks (p=0.147) (Figure 3.9).

There was a significant main effect of MW on peak rise (p<0.05), where H and N elicited the lowest and highest peak rise values respectively (Figure 3.8 (b)). No main effect of volume was found (p=0.718) (Figure 3.8 (c)).
Figure 3.8  (a) Mean peak rise elicited by test foods. Values are mean ± SEM (n=15). (b) Main effect of MW on mean peak rise. Each value is the mean peak rise elicited by foods of a single MW level. Values are mean ± SEM (n=30). (c) No main effect of volume on mean peak rise. Each value is the mean peak rise elicited by foods of a single volume level. Values are mean ± SEM (n=45). Bars with different letters differ significantly (p<0.05).
Figure 3.9  (a) Mean AUC for each test food. Values are mean ± SEM (n=15). (b) No main effect of MW on AUC. Each value is the mean AUC elicited by foods of a single MW level (n=30). Values are mean ± SEM (c) No main effect of volume on AUC. Each value is the mean AUC elicited by foods of a single volume level (n=45). Values are mean ± SEM. Bars with the same letters are not significantly different (p<0.05).
Figure 3.10 shows the effect of β-glucan on peak rise in more detail. The 250H and 600H solutions had the same dose and MW of β-glucan fibre but different volume. This difference in volume produced a difference in viscosity, but the peak rise did not differ between the two solutions. This was also found for the 600L and 250L solutions.

In contrast, the 250L and 600H solutions which were concocted to have similar viscosities but different volume and MW differed significantly in their effect on peak rise (p<0.05). The 600H solution containing high MW β-glucan elicited a lower peak rise than the 250L solution containing low MW β-glucan.

The glucose-only solutions had zero viscosity and resulted in the highest peak rise values. The 600N solution gave a slightly higher peak rise than the 250N solution, but this difference was not significant.

Figure 3.10  The effect of β-glucan solutions of different viscosity on peak rise. Value are mean ± SEM. Data points with different letters differ significantly (p<0.05).
3.4 DISCUSSION

β-glucan solution viscosity was successfully manipulated by varying the β-glucan MW and varying the solution volume. High solution viscosity was achieved with high β-glucan MW or with low solution volume. The results follow the established relationship between the log (viscosity) and log (C x MW) of β-glucan solutions. When C is held constant in a β-glucan solution of fixed volume and dose, increasing or decreasing the MW respectively increases or decreases the solution viscosity. Similarly, when β-glucan MW and dose are fixed, the viscosity of the solution increases with decreasing volume and decreases with increasing volume.

The effect of β-glucan and volume are apparent when examining the blood glucose curves. The control curves (N) generally had a higher peak rise than the curves containing β-glucan, while blood glucose values for the β-glucan curves were higher than those of the control curves at the end of the sampling period. This finding, as well as the fact that β-glucan curves were broader and flatter than control curves, may be explained by the delayed release of glucose from the β-glucan solutions under digestion and delayed absorption of glucose into the bloodstream due to the presence of β-glucan solution viscosity. As a result, the rise and fall in blood glucose levels is more gradual, leading to an overall attenuated glycemic response when compared to glucose control.

The association between high β-glucan solution viscosity and low glycemic response was observed in this study. There was a high, negative correlation between peak rise and both log (viscosity) and log (C x MW). On the other hand, the correlation between log (viscosity) and log (C x MW) with AUC was weak and not significant. However, this does not necessarily suggest a lack of effect of β-glucan on lowering glycemic response, and
similar results have been reported elsewhere (9, 41). Since the mechanism for how β-glucan lowers blood glucose levels involves a more gradual release and absorption of glucose, the appearance of glucose in the blood is delayed. This results in an overall lower peak rise in blood glucose, as well as a more gradual return of glucose levels to fasting. The shape of the glycemic response curve elicited by a glucose solution containing β-glucan compared to that of a glucose-only control shows that the β-glucan curve is blunted at the maximum and is broader than the control curve. Essentially, the area under the β-glucan curve is similar to that of the glucose curve, whereas the shape of the two curves differs. Therefore, a lack of significant difference on AUC between the β-glucan drinks and the glucose-only drinks in this study demonstrates not the inefficacy of β-glucan on lowering blood glucose, but the strength that β-glucan has in eliciting a steadier rise and fall of blood sugar than glucose alone, and highlights its potential for assisting individuals in good glycemic control.

At the volumes used in this study (250ml and 600ml), changing viscosity by changing volume did not contribute to β-glucan bioactivity. This was evident in the detection of a main effect for MW but not for volume on peak rise. The fact that the change in viscosity affected peak rise only when viscosity was altered by MW and not by volume is a novel finding. Although log (viscosity) was negatively associated with peak rise, the results of this study suggest that the physical (rather than rheological) properties of β-glucan hold importance in how this soluble fibre affects glycemic response. Previous studies have demonstrated that increasing viscosity by increasing β-glucan MW or C can strengthen blood glucose-lowering, but these studies increased C by increasing β-glucan dose without changing solution volume. Thus, while a higher β-glucan C, and thus higher viscosity, achieved a greater lowering of glycemic response, it is unclear whether this was due to an increase in viscosity or an effect
of dose-response. Since our study found that solutions with the same β-glucan MW and dose may yield similar effects on peak rise when the viscosity of these solution differ, viscosity may not play as important a role in β-glucan bioactivity as is believed. Moreover, β-glucan dose and MW may be better predictors of how β-glucan affects glycemic response. Thus, while the relationship stating that log (viscosity) is proportional to log (C x MW) still holds true, the inverse linear relationship between log (viscosity) and log (peak rise) is strongest when C is varied by dose at a constant solution volume. In other words, it is more accurate to state that the log (viscosity) of a β-glucan solution is inversely related to peak rise when log (viscosity) is directly proportional to log (dose x MW) rather than log (C x MW).

Although our results show that volume does not contribute to the effects of β-glucan on glycemic response, this does not dictate that volume itself, irrespective of β-glucan, has no effect on blood glucose levels. Indeed, food volume has been shown to affect gastric emptying, which has implications for glycemic response. Sievenpiper et al. (79) observed that increasing the volume of glucose solutions three-fold lead to a faster and higher rise in glycemic response. Young et al. (84) also found that a higher food volume resulted in changes to curve patterning, where peak rise increased with increasing volume up to 750ml. The authors explain that a higher volume may initially lead to a higher rate of glucose clearance from the gut, resulting in a greater initial rise and peak in glycemic response.

In our study, the effects of volume are apparent in the blood glucose curves and are similar to those of the studies discussed. A steeper rise and higher peak was observed when volume was 600ml compared to the lower 250ml volume. Although differences between 600ml and 250ml curves at each time point were mainly not significant, the larger volume of 600ml tended to yield a steeper rise and higher peak in blood glucose than the lower volume
of 250ml. The lack of significance in this case may be because 250ml and 600ml are not sufficiently different volumes. Where β-glucan is present, the lack of significance may be due to the suppression of glycemic response by β-glucan, which could result in both curves being attenuated to the point where there is little difference in blood glucose concentration between the curves at individual time points.

3.5 CONCLUSIONS AND FUTURE EXPERIMENTS

This experiment sought whether altering β-glucan solution viscosity by altering volume changes the effect of β-glucan on glycemic response. We found MW and not volume to be the main contributing factor on how β-glucan affects blood glucose levels. However, volume itself, irrespective of β-glucan, affects the patterning of the glycemic response curve. Therefore, the presence of β-glucan and the type of β-glucan remain most important for lowering glycemic response.

Since our study only tested volume at 250ml and 600ml, the results may not apply to other volume levels. Future experiments should test a broader set of solution volumes in order to determine the range of volumes to which our results hold true. This would help answer related questions, such as how large a volume is necessary in order for the effect of volume on gastric emptying to eclipse the lowering effect of β-glucan, and conversely, at what level of β-glucan concentration will allow for the effect of β-glucan to overcome the effect of volume on gastric emptying.
CHAPTER 4

EXPERIMENT 2:

COMPARISON OF THE EFFECTS OF β-GLUCAN IN SOLUTION WITH β-GLUCAN IN THE FORM OF A HARD GEL ON GLYCEMIC RESPONSE
4.1 OBJECTIVES

To examine how a solid β-glucan hard gel affects glycemic response \textit{in vivo} compared to β-glucan in solution, and to determine whether glucose diffusion from solutions and gel \textit{in vitro} relate to glycemic response results ascertained \textit{in vivo}.

4.2 MATERIALS AND METHODS

4.2.1 Characterization of test foods and \textit{in vivo} glycemic response determination

Refer to Chapter 2, Sections 2.1-2.6.

4.2.2 \textit{In vitro} glucose diffusion analysis

Refer to Chapter 2, Section 2.7.

4.3 RESULTS

4.3.1 Physiochemical properties of β-glucan drinks and gel

Values for the doses of low and high MW β-glucan, physical state (liquid or gel), volume, concentration of low MW β-glucan, and β-glucan MW for the foods are summarized in Table 4.1.

<table>
<thead>
<tr>
<th>Food</th>
<th>Physical State</th>
<th>Low MW β-glucan (g)</th>
<th>High MW β-glucan (g)</th>
<th>Volume (L)</th>
<th>C of LMW β-glucan (g/L)</th>
<th>MW (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>130N</td>
<td>liquid</td>
<td>0</td>
<td>0</td>
<td>0.130</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>130L</td>
<td>liquid</td>
<td>4.00</td>
<td>0</td>
<td>0.130</td>
<td>30.8</td>
<td>1.45 x 10^5</td>
</tr>
<tr>
<td>130H</td>
<td>liquid</td>
<td>0</td>
<td>4.00</td>
<td>0.130</td>
<td>0</td>
<td>5.80 x 10^5</td>
</tr>
<tr>
<td>LMWG</td>
<td>gel</td>
<td>4.00</td>
<td>0</td>
<td>0.130</td>
<td>30.8</td>
<td>1.45 x 10^5</td>
</tr>
</tbody>
</table>

The viscoelasticity of the foods was measured and is represented by values of the storage modulus ($G'$) and loss modulus ($G''$) (, descriptors of the solid-like and liquid-like behaviours of the food respectively,) over a range of strain frequencies (0.1 – 100s$^{-1}$). $G'$ and
G” curves for the foods are shown in Figures 4.1 and 4.2. As frequency increased, G’ for 130H increased and eventually exceeded G”. While both G’ and G” of 130L increased with increasing frequency, G” dominated throughout the strain period. For LMWG, G” increased with increasing frequency. However, G” did not surpass G’ by the end of the strain period, and G’ itself remained constant over the frequency range.

General observations on the physical properties of the foods were made. 130H had the thickest consistency of the β-glucan solutions, and the solid gel LMWG was brittle.
4.3.2. Effect of isovolumetric β-glucan solutions and gels on glycemic response

The glycemic response curves elicited by the drinks and β-glucan hard gel are shown in Figure 4.3. 130N (containing only glucose) and 130H (containing glucose and high MW β-glucan) appeared to elicit the highest and lowest glycemic responses respectively. Blood glucose concentration for all β-glucan-containing foods was significantly lower than that of 130N for at least one time point in the sampling period (p<0.05).

In order to determine whether β-glucan solutions differ from β-glucan gels in their effect on glycemic response, comparisons were made between the two foods which were identical in composition but different in their physical state of matter. Since 130L and LMWG are identical in all respects except that one is a liquid and the other a gel, comparisons between these two foods are of interest. Figure 4.3 shows that 130L achieved a lower glycemic response than LMWG, and that the curve of LMWG is similar to that of the glucose control 130N.
Differences between the peak rise (Figure 4.4) and the incremental area under the glycemic response curve (AUC) (Figure 4.5) of the foods were observed. For the solutions, both AUC and peak rise decreased as MW increased, with 130L and 130H having significantly lower values than 130N (p<0.05). Furthermore, 130H had lower AUC and peak rise values than 130L, although these differences were not significant. As for the comparison between low MW solution and gel, both AUC and peak rise for 130L were lower than those of LMWG, with the difference in peak rise reaching significance (p<0.05).

**Figure 4.3** Glycemic response curves elicited by test foods. Data points with different letters are significantly different (p<0.05). Values are mean ± SEM.
**Figure 4.4** Effect of test foods on mean peak rise. Bars with different letters are significantly different (p<0.05). Values are mean ± SEM.

**Figure 4.5** Effect of test foods on AUC. Bars with different letters are significantly different (p<0.05). Values are mean ± SEM.
4.3.3  *In vitro* glucose diffusion analysis

The amount of glucose released from samples of 130N, 130L, 130H and LMWG at every 15 minutes over a 2-hour period were measured *in vitro*. Values are expressed as a percentage of the starting amount of glucose in the dialysis bag, and were determined by averaging the percentage of glucose diffused from 8 water samples at each time point (Table 4.2).

The percentage of total glucose diffused from each food was plotted against time ([Figure 4.6](#)). The percentage of glucose released from 130N was greater than that of the other foods at every time point (p<0.05). Glucose diffusion from LMWG was the lowest of the foods at each time point (p<0.05), while glucose release from 130L and 130H did not differ significantly at any point in time.

The final percentage of total glucose released from each food was taken as the percentage of glucose released at 120 minutes (Table 4.2). The final percentage was lowest for LMWG and highest for 130N, with the final percentages for 130H and 130L being intermediate ([Figure 4.7](#)). The percentage values for both LMWG and 130N differed significantly from that of the other foods (p<0.05). Percentages for 130H and 130L were similar (p>0.05).
Table 4.2  Percentage of glucose diffused from test foods *in vitro* over 120 minutes at 37°C†.

<table>
<thead>
<tr>
<th>Food</th>
<th>Time (minutes)</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>75</th>
<th>90</th>
<th>105</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>130N</td>
<td>0</td>
<td>± 28.6</td>
<td>± 44.8</td>
<td>± 56.8</td>
<td>± 64.8</td>
<td>± 70.6</td>
<td>± 75.1</td>
<td>± 78.4</td>
<td>± 80.8</td>
<td>± 1.4</td>
</tr>
<tr>
<td>130L</td>
<td>0</td>
<td>± 18.8</td>
<td>± 28.6</td>
<td>± 36.1</td>
<td>± 41.7</td>
<td>± 47.6</td>
<td>± 51.1</td>
<td>± 55.1</td>
<td>± 58.5</td>
<td>± 0.5</td>
</tr>
<tr>
<td>130H</td>
<td>0</td>
<td>± 18.3</td>
<td>± 28.3</td>
<td>± 35.9</td>
<td>± 43.7</td>
<td>± 49.9</td>
<td>± 55.6</td>
<td>± 60.4</td>
<td>± 64.8</td>
<td>± 0.9</td>
</tr>
<tr>
<td>LMWG</td>
<td>0</td>
<td>± 12.5</td>
<td>± 20.8</td>
<td>± 27.3</td>
<td>± 32.6</td>
<td>± 37.5</td>
<td>± 41.5</td>
<td>± 45.2</td>
<td>± 49.2</td>
<td>± 0.4</td>
</tr>
</tbody>
</table>

† Values are the mean of percentages from eight water samples ± SEM.

Figure 4.6  Percentage of total glucose diffused over time from β-glucan solutions and gel *in vitro*. Data points with different letters are significantly different (p<0.05). Values are mean ± SEM.
Figure 4.7  Final percentage of total glucose diffused from foods after 120 minutes. Bars with different letters are significantly different (p<0.05). Values are mean ± SEM.
4.3.4. **Comparison of *in vitro* data with *in vivo* glycemic response results**

The final percentage of glucose diffused from foods containing β-glucan was negatively correlated with *in vivo* peak rise ($r^2=0.997$, p=0.036, **Figure 4.8**) and AUC ($r^2=0.998$, p=0.030, **Figure 4.9**). 130N was not included in the regression analysis because it did not contain β-glucan.

**Figure 4.8**  The regression relationship between peak rise in blood glucose concentration elicited by foods and the final percentage total glucose diffused from foods *in vitro* after 120 minutes. Values are mean ± SEM.
Regression equation is in the form: \( \text{variable} = a + b \times \text{Final % Glucose Released} \)  

\( \text{SEE} \) = standard error of the estimate of ‘a’ and ‘b’  
\( r^2 \) = correlation coefficient  
* Significance of the regression relationship

**Figure 4.9** The regression relationship between AUC elicited by foods and the final percentage total glucose diffused from foods *in vitro* after 120 minutes. Values are mean ± SEM.

**Table 4.3** The regression relationship between Final % Glucose Released *in vitro* after 120 minutes and glycemic response results obtained *in vivo*.

<table>
<thead>
<tr>
<th>Variable</th>
<th>a</th>
<th>SEE</th>
<th>b</th>
<th>SEE</th>
<th>( r^2 )</th>
<th>( p^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Rise</td>
<td>7.71</td>
<td>0.253</td>
<td>-0.077</td>
<td>4.38x10^{-3}</td>
<td>0.997</td>
<td>0.036</td>
</tr>
<tr>
<td>AUC</td>
<td>4.96x10^2</td>
<td>13.9</td>
<td>-5.14</td>
<td>0.241</td>
<td>0.998</td>
<td>0.0298</td>
</tr>
</tbody>
</table>

Regression equation is in the form: variable = a + b \[\text{Final % Glucose Released}\]

\( \text{SEE} \) = standard error of the estimate of ‘a’ and ‘b’  
\( r^2 \) = correlation coefficient  
* Significance of the regression relationship
4.4 DISCUSSION

The differences between the physical properties of the β-glucan solutions and hard gel were verified by viscoelastic measurements. As constant strain was applied at increasing frequency, the rise in $G'$ to exceed $G''$ for 130H but not for 130L confirms that 130H is a highly viscous solution compared to 130L. The rapid rise in $G'$ and higher $G'$ value than $G''$ is the behaviour typically observed with fibre solutions of high viscosity (2, 27). For LMWG, the fact that the $G'$ value was stable throughout the strain period indicates that LMWG was indeed a solid gel.

With regards to the in vivo glycemic response elicited by the foods, β-glucan bioactivity was observed for both AUC and peak rise, but differences between peak rise values were more prominent than differences between AUC values. This, however, is a commonly observed effect of viscous soluble fibres on glycemic response (refer to Experiment1) and should not be regarded as a lack of effect on blood glucose-lowering. In fact, the effect of β-glucan is clearly demonstrated in the curves (Figure 4.3), as the presence of β-glucan in LMWG, 130L and 130H diminished blood glucose values compared to the control 130N. The greatest differences in glycemic response from 130N were achieved by 130H, indicating that high MW is important for glycemic response-lowering. The effect of β-glucan is also evident in that the foods containing β-glucan tended to yield higher (though not significantly higher) blood glucose values than the control at the end of the sampling period, which suggests that β-glucan foods elicited a more gradual lowering of glucose levels after peak rise had been reached (Figure 4.3).

In order to determine whether β-glucan solutions differ from β-glucan gels in their effect on glycemic response, 130L and LMWG in particular were compared against each
other. For both peak rise and AUC, 130L gave significantly lower values than LMWG, despite having the same amount and type of β-glucan. Thus, the results of our study show that, at least when low MW β-glucan is used, β-glucan gels are not effective at lowering glycemic response compared to β-glucan solutions.

The *in vitro* glucose diffusion from β-glucan solutions and gel was also examined to determine the relationship between the release of glucose from the foods and the observed *in vivo* glycemic response. The analysis revealed that a lower total percentage of glucose diffused *in vitro* corresponded to a greater peak rise and AUC. These results appear contradictory because a lower glycemic response would be expected if less glucose is released from food. However, the results of the glucose diffusion analysis must be interpreted carefully, as it measures the capacity for glucose to be retained in the food and does not take into consideration any conditions which are external to the food matrix. For example, while the analysis showed that glucose diffusion from LMWG was the lowest of the foods and that therefore the gel structure may effectively retain glucose, it does not necessarily reflect the efficacy of the gel to retain glucose *in vivo*. Indeed, the diffusion analysis protocol was not designed to mimic the gut environment where the foods would be digested, as there was no inclusion of digestive enzymes, no adjustment of pH to simulate that of the gut, nor was there any mimicking of mechanical digestion which would normally occur in the body. It is possible that under mechanical digestion, the brittle nature of the LMWG would have caused the gel to break apart and allow glucose to easily diffuse into the gut, making the gel no more effective than the glucose-only solution 130N at attenuating glycemic response. Furthermore, it is possible that β-glucan must be in solution in the gut upon consumption in order for glycemic response-lowering to take effect. It has been observed that β-glucan gels do not
melt below 60°C (2, 25, 27), and indeed, LMWG did not liquefy in the water bath that was set to maintain the physiological temperature of 37°C in the in vitro experiment. Therefore, the β-glucan in LMWG may have remained immobile in the gel matrix, which would prevent proper solubilization of β-glucan in the liquid digestive contents. According to the proposed mechanism that is currently accepted, it is the viscosity of β-glucan solutions which form in the gut that lead to a lower postprandial glycemic response. If this is true, then the β-glucan in LMWG would not have been bioactive because it would not have lead to viscosity development. Referring to the results of Experiment 1 however, viscosity may not play as important a role in bioactivity as believed. Whether or not viscosity is a critical factor for β-glucan to elicit physiological benefits, it is likely that β-glucan must at least be solubilized in the gut in order to affect glycemic response. This may be more achievable if β-glucan is already dissolved in solution prior to consumption than if β-glucan is ingested as a solid food. The effect of the gel matrix on β-glucan bioactivity requires further investigation.

While our study found gel form not to be important, MW again was a critical determinant of how β-glucan affects glycemic response. As MW increased among the solutions, both peak rise and AUC decreased.

4.5 CONCLUSIONS AND FUTURE EXPERIMENTS

In conclusion, our study suggests that although low MW β-glucan forms stronger hard gels and gel more efficiently than high MW β-glucan (25, 27), low MW β-glucan does not lower glycemic response as it would in solution.

Our study did not investigate the effects of a gel consisting solely of high MW β-glucan because the time required for this gel to form would well exceed the amount of time needed to form a low MW gel. It has been observed that high MW β-glucans do not gel even
after 7 days (2, 25, 27), and there is a risk that the structure of a low MW gel would
deteriorate by the time a high MW gel has come to form. Moreover, it would not be practical
to create high MW gels that do not form efficiently, despite that high MW polymers are most
effective at lowering glycemic response.

The in vitro analysis showed that although there was a greater retention of glucose in
β-glucan gel than β-glucan solutions, the conditions to which foods are exposed when
digested in vivo, and the properties of the food matrix itself, ultimately determine how
glucose is released from the foods. For future analyses of glucose diffusion, an in vitro
digestion model that recreates the conditions of the gut environment could be used, and these
results could be compared to clinical data. The negative association between the glucose
diffusion data and glycemic response data illustrates the importance of verifying results from
in vitro experiments with those from in vivo experiments, since it is the true physiological
effects which are clinically relevant.
CHAPTER 5

FINAL CONCLUSIONS
5.1 FINAL CONCLUSIONS

The glycemic response elicited by β-glucan with different physical properties and food form were investigated through two novel experiments.

In Experiment 1, β-glucan solution viscosity was altered by changing the solution volume at a constant β-glucan dose. Since two solutions with the same β-glucan dose and molecular weight but different viscosity did not yield differences in glycemic response, the long-established association between β-glucan solution viscosity and glycemic response-lowering may be challenged. In addition, an effect of solution volume on glycemic response patterning was observed, where a higher solution volume tended to result in a faster and higher rise in blood glucose levels. Thus, these results show that a solution high in β-glucan dose and low in solution volume may be most effective at attenuating glycemic response. Further studies must be performed to verify the results attained here.

In Experiment 2, a low molecular weight β-glucan hard gel was tested for its effect on glycemic response. This experiment showed that the β-glucan gel, unlike β-glucan in solution, is incapable of lowering blood sugar levels. The in vitro glucose diffusion analysis revealed that β-glucan in gel form has a greater potential to retain glucose than β-glucan solutions, but this does not manifest as a reduction in glycemic response in vivo. Thus, this experiment demonstrated the importance of β-glucan administered in liquid form for therapeutic purposes, as well as the importance of clinical testing when investigating the health effects of functional foods.

Overall, the results of this study will be useful in the development of β-glucan foods that may help in glycemic control, but there are limitations to our study and the use of β-glucan as a functional food ingredient. Firstly, it is possible that a sufficiently high dose of β-
glucan must be consumed in one sitting, rather than portioned for consumption throughout
the day, in order for there to be a glycemic response-lowering effect. This is because low β-
glucan doses may hamper bioactivity (63). However, Acheson et al. (63) showed that β-
glucan administered in small doses throughout the day lowers glycemic response through the
second meal effect. This effect suggests that consuming a meal containing low-GI foods or
foods that lower glycemic response such as β-glucan, may suppress the postprandial
glycemic response to a subsequent meal (10). Future studies should compare the glycemic
response-lowering effect of β-glucan consumed in small quantities over the course of the day
to that of β-glucan consumed in a single large quantity. This would help determine the
appropriate β-glucan dose to use in functional foods and how effectively small doses of β-
glucan reduce glycemic response when served at several meals during the day. Another
concern with using β-glucan in functional foods is the palatability of the product. In our
study, participants made informal comments about the high viscosity β-glucan drinks being
difficult to swallow. The highest viscosity drinks containing high molecular weight β-glucan
were the most effective at lowering glycemic response, but problems with palatability may
limit the clinical utility of this food. Increasing palatability of β-glucan foods will require
solutions that heighten product acceptability without compromising bioactivity.

With the incidence of diabetes reaching epidemic proportions and without a cure for
this disease, prevention and management are vital for the health of the population. The ability
for β-glucan to attenuate glycemic response gives it dual functionality as both a preventative
and management tool for diabetes, which warrants further investigation on the physical
properties responsible for β-glucan bioactivity and the food form in which β-glucan
effectively lowers glycemic response.


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