Physiological Effects of Barley: Examining the Effects of Cultivar, Processing and Food Form on Glycemia, Glycemic Index, Satiety and the Physico-Chemical Properties of β-glucan

by

Ahmed Aldughpassi

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

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Barley has been receiving increased attention as a human food due to the health benefits associated with β-glucan fiber and its potential as a low glycemic index (GI) functional food. Research has shown a relationship between the physico-chemical properties of β-glucan and the physiological effects, which may be altered by processing. However, it is not known if the physiological effects of consuming barley are affected by variations in chemical composition among cultivars or by common processing methods such as pearling or milling. The primary objective of this thesis was to characterize the effects of differences in cultivar starch and fibre content, level of pearling and milling on the GI, satiety and the physico-chemical properties of β-glucan. Nine barley cultivars varying in starch-type and β-glucan content were studied in three experiments in separate groups of ten healthy participants. Blood glucose and satiety ratings were measured and the GI was calculated. Total starch, total fibre, β-glucan, molecular weight (MW), solubility and β-glucan viscosity were determined in vitro. Results showed that GI varied by cultivar (CDC-Fibar, 26 ± 3 vs. AC-Parkhill, 35 ± 4, P < 0.05) and pearling (WG, 26 ± 4 vs. WP 35 ± 3, P < 0.05). When two cultivars were milled and processed to wet pasta the GI increased by 184% (P < 0.05). The pearled wet pasta had a significantly lower GI compared to the whole grain (P < 0.05). Boiled barley kernels tended to elicit greater satiety than white bread, but the difference was not significant. In both the boiled barley kernels and the wet pasta, pearling did not affect the MW, viscosity and solubility. MW did not significantly differ between cultivars but solubility and viscosity did (P < 0.05). The wet pasta had significantly lower MW, solubility, viscosity but not β-glucan content than the boiled barley kernels (P < 0.05). In conclusion, pearling did not have an effect but milling and extruding resulted in significant reduction in MW, solubility and viscosity. The GI of barley is influenced significantly by cultivar, pearling and milling. Further studies are required to determine the effect on satiety.
Acknowledgment

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<tr>
<td>AACC</td>
<td>American Association of Cereal Chemists International</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BG</td>
<td>Blood Glucose</td>
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<td>CFG</td>
<td>Canada Food Guide</td>
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<tr>
<td>CHO</td>
<td>Carbohydrates</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
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<tr>
<td>CP</td>
<td>Commercially Pearled</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<tr>
<td>db</td>
<td>Dry Basis</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
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<tr>
<td>FBG</td>
<td>Fasting Blood Glucose</td>
</tr>
<tr>
<td>FDA</td>
<td>The Food and Drug Administration</td>
</tr>
<tr>
<td>FIA</td>
<td>flow-injection analysis</td>
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<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GI</td>
<td>Glycemic Index</td>
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<tr>
<td>HbA1c</td>
<td>Glycated Hemoglobin</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental Area Under the Curve</td>
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<tr>
<td>L</td>
<td>Liter</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein cholesterol</td>
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<tr>
<td>Min</td>
<td>Minutes</td>
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<tr>
<td>ml</td>
<td>Milliliter</td>
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<tr>
<td>MW</td>
<td>Molecular Weight</td>
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<td>PP</td>
<td>Pot Pearled</td>
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<tr>
<td>PRBG</td>
<td>Peak Rise Blood Glucose</td>
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<tr>
<td>RAG</td>
<td>Rapidly Available Glucose</td>
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<tr>
<td>RCT</td>
<td>Randomized Clinical Trial</td>
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<tr>
<td>RDS</td>
<td>Rapidly Digested Starch</td>
</tr>
<tr>
<td>RR</td>
<td>Relative Risk</td>
</tr>
<tr>
<td>RS</td>
<td>Resistant Starch</td>
</tr>
<tr>
<td>RVA</td>
<td>Rapid Visco Analyzer</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>SDS</td>
<td>Slowly Digested Starch</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>SI</td>
<td>Satiety Index</td>
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<tr>
<td>Type2DM</td>
<td>Type 2 Diabetes Mellitus</td>
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<tr>
<td>WB</td>
<td>White Bread</td>
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<tr>
<td>WG</td>
<td>Whole Grain</td>
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<td>WHO</td>
<td>World Health Organization</td>
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1. Introduction

Carbohydrates (CHO) are omnipresent in our diets representing a major source of energy for humans. They typically account for 45-70% of our total energy intake\(^1\). Traditionally, carbohydrates have been classified into two categories: ‘simple’ or ‘complex’\(^2\). However, a major concern with this classification is the lack of ability to predict plasma blood glucose and insulin responses\(^3\), which are thought to be crucial factors in the etiology of many health complications. Type 2 diabetes (T2D), coronary heart disease, metabolic syndrome and cancer are highly prevalent chronic illness where carbohydrates have an underlying pathophysiological relevance\(^4, 5\). Yet, the source and type of CHO for optimal health and disease prevention is continually debated\(^6\).

Despite the lack of consensus on the optimal CHO source, type and amount\(^7\), there is wide agreement on the importance of whole grains (WG) and their beneficial effects in preventing weight gain and illness such as (T2D) and cardiovascular disease (CVD)\(^8\). Other than being a dominant source of carbohydrates in our diet, cereal grains contain a myriad of beneficial nutrients and phytochemicals that can be lost during processing\(^9\). Not all grains are equal, the health benefits seen from consuming one whole grain may not necessarily reflect the same magnitude of benefits from another whole grain. There is a wide diversity in the chemical composition between grains, in particular in starch and type of fibre, which in turn dictate the physico-chemical characteristics and their health promotion capacity.

Likewise, there is an abundance of evidence indicating that the type and quality of CHO plays a significant role with respect to health, disease prevention and management
A physiological measure of the quality of CHO, the Glycemic Index (GI), which classifies CHO rich foods based on their impact on glycemia, has been well established with respect to its importance in assessing CHO quality\(^{(12, 13)}\). Low-GI foods and diets have been repeatedly found to produce favorable effects on a number of physiological parameters including: postprandial glycemia in normal and T2D individuals\(^{(14, 15)}\), glycated hemoglobin (HbA\(_1c\))\(^{(16)}\), lipids\(^{(17)}\), and inflammatory markers\(^{(18)}\). Consequently, these effects result in reducing the risk of developing T2D, CVD and metabolic syndrome\(^{(15)}\).

In the latest Health Canada’s food guide, released in 2007\(^{(19)}\) and the 2010 U.S. dietary Guidelines\(^{(20)}\), eating whole grain foods was highly encouraged. Although this advice seems appropriate because most whole grains contain high levels of important nutrients such as dietary fibre and magnesium, not all sources of whole grains or Fibre are equal with respect to their physiological effects\(^{(21)}\). On one hand, because of their varied chemical composition and physical form the rate and degree to which the different types of whole grains are digested and absorbed from the small intestine varies. On the other hand, whole grains that can be processed and reconstituted into a variety of different forms, will not necessarily have a low GI and may not exert the same benefits as the original whole grains. Consequently, these differences may lead to unfavorable effects on glycemia, GI and satiety. This puts forward the need for improved methods of how we classify whole grains.

Epidemiological studies repeatedly show that whole grains are protective against a number of chronic diseases and weight gain\(^{(8)}\), yet data are scarce in distinguishing between the different whole grains and exploring the biological mechanisms responsible
for these effects. Fibre has been extensively examined as a key player in the favorable effects incurred by whole grains but this is not conclusive (22). Other factors such as chemical composition, processing and food form may have a significant role in this relationship, yet they are not copiously investigated when considering the quality of whole grains. It is still debated whether all whole grains are equally protective and how consumers can easily distinguish friend from foe when choosing a whole grain food product. A recent report examining the relationship of whole grains and CVD health-claims using the FDA definition of whole grains concluded that the health benefits seen from consuming one whole grain do not necessarily reflect the same magnitude of benefits from other whole grain (23). This has created interest in whole grains with intact kernels that are slowly digested, specifically barley.

Recently, United States Food and Drug Administration (FDA) approved a health claim for certain barley-containing products linking the ingestion of β-glucan with reduced risk of coronary heart disease (24). This has propagated wide interest in barley among consumers and subsequently industry has responded with a wide range of β-glucan and barley-containing products. Similarly, Health Canada has also approved a food health claim for barley in 2012 (25). Despite this, barley is not extensively consumed in North America compared to oats or other cereals, with the average individual consuming approximately 0.5 kg/year/person (26). On the other hand, the desire to develop palatable barley based products raises a number of concerns regarding the impact of processing and refinement of barley on physiological parameters such as glycemia, GI and subjective satiety.

Elucidation of the role of whole grains quality in health promotion requires a
thorough understanding of the physico-chemical properties of food and processing methods; in particular, pearling, a common commercial process whereby the husk and outer layers of barley grains are removed by a friction and abrasion process \(^{(27)}\). Factors such as the compositional diversity in barley cultivars, amylose-to-amylopectin ratio, solubility, viscosity, molecular weight (MW), food form and cooking may influence the post prandial responses of barley products, alter their GI and subsequently affect subjective satiety. Yet, these factors need to be examined.

There is a need to understand the contribution of the intact whole grains kernel vs. the processed and altered grain on the beneficial effects of barley. There are gaps in our knowledge with respect to the contributions of the physico-chemical properties to the positive effects of whole grains on metabolic risk factors such as glycemia. Whether single components of the grain are responsible for these effects or a synergistic effect exist is not known. For example, the beneficial effects of β-glucan on cholesterol lowering depend on its ability to increase viscosity in the small intestine, which in turn is determined by MW and solubility \(^{(28)}\). A different framework for measuring CHO quality in whole grains is needed to allow a greater understanding of individual species in health to inform the public of their efficacy.

When it comes to intact whole grains data are scarce with regards to the contribution of the aforementioned factors on glycemia, GI and subjective satiety. There is a necessity for a better understanding of the synergistic effects of whole grains constituents and processing on health. To reveal the contribution of chemical composition, processing, food form and physico-chemical properties of barley this thesis, using barley as a surrogate for intact whole grains foods, will attempt to answer the
following questions:

1. What is the significance of differences in chemical composition, total fibre and β-glucan content on glycemic response, GI and subjective satiety?
2. What is the effect of food form on glycemic response, GI and subjective satiety?
3. What is the magnitude and impact of processing and food form on the physico-chemical properties of barley?
4. What is the contribution of the physico-chemical properties with respect to glycemia and the GI?

In chapter 2, the historical background of whole grains on health and disease prevention with emphasis on barley and its role in human health is reviewed. Chapters 4 and 5 will represent the three in vivo randomized clinical trials (RCT) addressing the first three previously mentioned questions. Chapter 4 will assess the contribution of chemical composition including starch composition and its nature (slowly digested starch vs. rapidly digested starch), total fibre and β-glucan content, pearling and food form on glycemia and the GI of barley. Chapter 5 will address the effects of differences in chemical composition, pearling and food form on subjective satiety and the satiety index score (SI). Chapter 6 will investigate the contribution of the physico-chemical properties on glycemia and the GI; in particular, the impact of viscosity and molecular weight. In chapter 7 the significance and implications of this work is discussed with emphasis on identifying the integral aspects when considering the efficacy of barley and barley food products as low-GI foods.
2. LITERATURE REVIEW

2.1. Whole Grains

In order to appreciate the significance of investigating factors such as the compositional diversity in barley cultivars, solubility, viscosity, molecular weight (MW) (i.e physico-chemical properties), and food form on the efficacy of barley requires a review of whole grains literature to grasp the gaps in our knowledge. The work below will include a discussion of whole grains definition, or lack off. An attempt to underline the consequences of lack of consideration of the aforementioned factors on the efficacy of whole grains, barley and barley food products in previous studies including prospective cohorts and intervention studies is reviewed. A review of barley constituents, processing methods and significance to health and disease will also be undertaken. Finally, the significance of the glycemic index and how it relates to whole grains and barley will be highlighted.

According to the International Association for Cereal Science and Technology (ICC), and the European Health Grain Project, “the term ‘Grain’ applies to the genera and species of the grass family *Poaceae*”. Whole grains are whole cereals such as wheat, rice, maize, oats, barley and pseudocereals such as quinoa and amaranth. Grains that belong to the *Poaceae* family are related at the structural and biochemical levels, which also include pseudocereals (29).

Currently the most widely accepted definition, introduced over a decade ago, is that of the American Association of Cereal Chemists International (AACC). The AACC defines whole grains as consisting of the “Intact, ground, cracked or flaked caryopsis
[grain], whose principle components – the starchy endosperm, germ and bran – are present in the same relative proportions as they exist in the intact caryopsis” (30). This definition provides the basis for food labeling, dietary recommendations and food health claims in Canada, the U.S., and a number of European countries.

Most intact whole grains have to be processed before consumption which includes milling the grain into flour and less damaging processes such as flaking, cracking and rolling. These processes create flour for foods like bread or cookies and rolled oats or breakfast cereals. The metabolic consequences of such processing on the characteristics of whole grains and the effects of separating whole grain constituents during milling for a later recombination are underappreciated. Currently, the definition includes milled whole grains into flour and the product is still considered a whole grain, otherwise known as a whole grain food. Whether the intact whole grain kernel is nutritionally different than the milled whole grain flour and if it will sustain the same physiological effects is not clear.

The lack of a global standardized definition along with scarcity in data in differentiating whole grains that are intact kernels vs. whole grain foods may have resulted in less than optimal labeling standards (31). The current labeling standards promote a potentially misleading label on many whole grain products and causes confusion among consumer and clinicians. For example, Health Canada allows the labels “Whole Wheat” and “Whole Grain” to be used on products but they don’t mean the same thing and many consumers and clinician are not aware of such knowledge. The Canadian Food and Drug Regulation standards for whole wheat flour allows for the exclusion of 5% of the wheat grain which means a loss of about 70% of the germ and some of the bran (32), this permits the label “Whole Wheat” but not a “Whole Grain” because of the loss of
Similarly, the U.S. FDA defines a whole grain food as any product containing > 51% whole grain by weight per reference amount customarily consumed (RACC) per day. This definition provided the basis for the 1999 FDA claim which was modified in 2003 and allows the following statement: “Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers” (33). Similar claims are also seen in European countries like the United Kingdom and Sweden. However, in 2010 the European Food Safety Authority (EFSA) did not approve a similar whole grain health claim. According to ESFA expert panel whole grain foods including whole grain flour are defined differently across the European Union countries (EU). The panel decision was based on the inability to characterize the food constituent of a whole grain (34). Health Canada rejected a similar health claim for whole grains due to insufficient evidence from prospective cohort studies and clinical trials and notes that the benefits seen from consuming specific whole grains such as barley and oats cannot be extrapolated to other grains such as wheat, which is the dominant grain in many parts of the world (35).

Nonetheless, whole grains and whole grain foods provide a myriad of important nutrients and recommending their consumption is emphasized in many countries. The Canada Food Guide (CFG) recommends Canadians to consume half of their grains in the form of whole grains. Similar recommendations are seen in the U.S. Dietary Guidelines 2010. Despite this, whole grains consumption is very low worldwide with some exception in Scandinavia, Eastern Europe, North Africa, Japan and Tibet.
2.2. Whole Grains and Disease Prevention

2.2.1. Prospective cohort studies

Intact whole grains and whole grain foods have been associated with many health outcomes, from promoting gut health\(^{36}\) to reducing the risk of disease biomarkers and developing chronic illness such as T2D\(^{37}\), CVD\(^{38}\), blood pressure\(^{39}\), metabolic syndrome\(^{40}\) and cancer\(^{41}\). The bulk of evidence comes from prospective cohort studies providing robust indicators of the relationship between whole grains consumption and health outcomes. There have been a number of meta-analyses further augmenting these associations\(^{8, 42}\). Ye et al\(^{8}\) conducted a recent systematic review and a meta-analysis examining the association between both whole grains intake and the risk of T2D, CVD and weight gain. Ye and colleagues found that subjects who consume an average of 48 - 80 g/d of whole grains had a 26\% reduction in T2D, a 21\% reduction in CVD risk and consistently less weight gain (1.27 vs. 1.64 kg) during 8 – 13 y of follow up compared to those who rarely or never consume whole grains. The range of reduction in relative risk (RR) in a multivariable-adjusted model was between 14\% - 35\% for 6 studies comprised of 2,919,482 person-years of follow-up and 12\% - 31\% for 10 studies comprised of 4,336,411 person-years of follow-up for T2D and CVD respectively.

On the other hand, most of the observational studies examining the relationship between whole grains and health outcomes suffer from methodological difficulties that hinder their comparisons; the method for measuring whole grain intake varied among studies and in some cases may be imprecise. For example, dark bread is considered to be whole grain bread in many of these studies regardless of its chemical composition. There are other data that are based on a more rigorous approach; De Moura et al\(^{23}\) assessed the
effect of applying the FDA definition of whole grains on the precision of scientific evidence supporting the claims for risk reduction of CVD. This approach resulted in the exclusion of many studies with only five eligible studies. De Moura and colleagues concluded that when considering only studies that fulfilled the FDA definition of whole grains, there is insufficient scientific evidence to support the claim associating whole grains intake and the reduction in the risk of CVD. However, the claim is supported only when using a broader definition that included studies considering the intake of fibre rich bran and germ as well as the whole grain. De Moura further states that despite the similarities whole grains share as a class, there are significant differences among the individual grains not only in fibre content or bran but in the content of putative bioactive compounds and thus the diverse grains may have significantly different health benefits.

2.2.2. Randomized clinical trials

Despite the robust indications of a strong relationship between a wider definition of whole grains and health outcomes from prospective cohort studies, randomized clinical trials (RCT) have not demonstrated similar results on a number of metabolic risk factors of chronic illness such as T2D, CVD and metabolic syndrome. This is further complicated by the fact that majority of clinical studies of the effects of whole grains on T2D and/or CVD risk biomarkers were conducted using single grains such as oats and barley or products with functional fibre (43, 44), thus limiting their generalizability to all the different whole grains. There is a lack of evidence from dietary intervention studies with large number of subjects showing the benefits of whole grains and disease outcomes. Nonetheless, there is a few RCT’s with small numbers of subjects and short duration that provide some insight to the relationship between WG and health.
Pereira et al (45) conducted a 6 week intervention of 7 servings of WG/day compared with equivalent as refined grain in 11 overweight hyperinsulinemic men with all the foods being supplied during the study. Pereira and colleagues found a 10% reduction in fasting insulin concentration and improved insulin sensitivity measured by euglycemic hyperinsulinemic clamp, which is considered the gold standard in measuring insulin sensitivity. However, study by Andersson et al (46) found no change in peripheral insulin sensitivity, measured by a euglycemic hyperinsulinemic clamp, in 22 postmenopausal women and 8 men fed 112 g of WG/day compared with equivalent amount of refined grain for 6 weeks. More recently a large European RCT, the WHOLEHeart study, investigated the effects of CVD risk markers of substituting WG for refined grains. The study consisted of 316 non WG consumers randomized to three groups: control (no dietary intervention, intervention 1 (60g WG/d for 16 weeks) and intervention 2 (60g WG/d for 8 weeks followed by 120g WG/d for 8 weeks). The study findings showed no significant differences between groups in body mass index (BMI), percentage body fat, waist circumference, fasting plasma lipid profile, glucose, insulin, inflammatory markers and endothelial function (47). A possible explanation for the lack of effect may be attributed to the intervention choice of test foods, mostly hi-GI and glycemic load (GL) processed and reconstituted whole grain foods rather than intact kernels; a choice made based on a realistic reflection of whole grain products sold in the study location. In the De Moura (23) comprehensive review they assessed 15 interventional studies, seven of which only tested oats with one showing no effect on total cholesterol (TC), 1 barley only and the rest included brown rice and wheat. They note “that the positive effect of barley reported across different populations, gender and health
status adds strength to the evidence for a beneficial health effect of barley on plasma TC and LDL-C levels”.

Despite the consistent epidemiological evidence supporting the notion that whole grains substantially lower the risk of disease metabolic markers and chronic illness, evidence from intervention studies is conflicted. The evidence repeatedly indicates that certain whole grains are superior to others in promoting health outcomes, in particular barley.

### 2.3. Barley

Barley, *Hordeum vulgare vulgar L.*, is an ancient grain with cultivation dating back to 8000 BC in the fertile crescent in the Middle East (48). In North America barley was mainly used as a human food but in the 19th and 20th centuries it evolved largely into an animal feed, malting and brewing grain. These uses drastically reduced barley human consumption, due in part to improved conditions of wheat production, along with the increase use of rice and maize in the human diet. Yet, barley is still a staple food in many parts of the world including Asia, Middle East, North Africa and Eastern Europe (49). In Tibet barley provides 80% of the calories in the diet of rural Tibetans (26). Similarly, in Morocco the average person consumes 68.3 kg of barley a year (50). In comparison, most individuals in the North America and Europe consume less than 1 kg/person/year with Canadians eating approximately 0.5 kg/year. In Canada, between 55 and 60% of barley is used as animal feed, 25-30% for brewing, 2-5% for seed and 2-5% for human consumption (51).

Barley is an important crop ranking fifth among all crops in dry matter production
in the world (52). In Canada, barley ranks third in grain production after wheat and maize. In the 2010/2011 market year, Canada ranked fifth worldwide in total barley production with 7,755,700 metric tons, and was responsible for 22% of the barley exported worldwide.

2.3.1. Barley cultivars

Barley may be one of the most widely adaptable grains allowing it to be cultivated in contrasting climates and various locations worldwide (53); it is a genetically diverse grain. This genetic diversity allows barley to be classified as either spring or winter types. Barley is further categorized as either a two-row or six-row; and hulled or hull-less. Two-row barley has 2 rows of seeds on each spike and six-row has 6 rows of seeds on each spike (botanically: two-row has 1 fertile floret per rachis node and a six-row has 3 fertile florets per rachis node) (48). Hulled and hull-less barleys are distinguished by the presence or absence of a hull tightly wrapping the grain. Any of these types can be further classified into either malting or feed barley depending on the end-use of the grain. Kernels from two-row barleys are generally larger and more uniform in size than those from six-rowed barleys due to crowding of spikelet’s on the spike in the latter. Hull-less barley are free threshing or naked grains. According to the grain chemical composition barley grains are further classified as normal, waxy, high amylose starch type, high lysine, and high β-glucan (54).

The physical and chemical characteristics of barley are an important aspect to be considered to reinstate barley as a human food. These aspects can be affected by processing, a necessary step in preparing barley for human consumption. The most common barley processing method is pearling, a common commercial process whereby
the husk and outer layers of barley grains are removed by a friction and abrasion. There has been a general preference, by consumers and food manufacturers alike, for a bright white colour of pearled barley and milled barley flour. Yet, in recent years the rising interest in whole grains and their products such as whole grain flour by consumers has diminished the demand for white food products such as white bread and pasta.

Figure 1. Barley grain with enlarged cross section.
2.3.2. Chemical composition

Barley cultivars vary widely in their chemical composition due to differences in genotype, growing environment and the interaction between the two (55). Normal barley generally consists of approximately 60 – 70% starch per dry matter (dm), making starch the most abundant constituent and found mostly in the endosperm (56). The next chief constituents are total fibre ranging from 11 – 34% and protein 10 – 20%; of total fibre 3 – 20% is soluble dietary fibre with 5 – 10% β-glucan depending on the cultivar. Other constituents include 2 – 3% free lipids and 1.5 – 2.5% minerals (57). Barley also contains a myriad of other components including a number of antioxidants and phenolic compounds (58), however, it is beyond the scope of this thesis and for that reason this review will discuss only the chief components of barley.

2.3.3. Available carbohydrates

In general, barley is predominantly composed of glycemic carbohydrates and dietary fibre. There is a small concentration of low molecular weight carbohydrates which must be included with starch, the predominant constituent, when calculating the composition of glycemic carbohydrates in barley. Barley has a small percentage of simple sugars like glucose, fructose, sucrose, maltose and raffinose (range 0.03 – 0.83 % db). Starch is the predominant glycemic carbohydrates in barley; in normal barley starch consists of amylose and amyllopectin polymers. Amylose is an essentially linear molecule consisting of glucose monomers joined by α 1-4 bonds. In contrast amyllopectin is a branched α 1-4 and α 1-6 linked molecules (56). The content of amylose in barley depends on the barley type with normal barley starch consisting of approximately 1:3 ratio of amylose to amyllopectin while waxy barleys can range from 0-5% amylose of total starch
The rate of glucose release into the blood stream from different starches can vary significantly \(^{(2)}\). Therefore for physiological and nutritional purposes starch can be further classified depending on its rate of glucose release into rapidly digested starch (RDS) and slowly digested starch (SDS). This classification is based on \textit{in vitro} measurements by Englyst methods \(^{(2)}\). RDS represent the amount of glucose released within the initial 20 min of digestion, whereas SDS represents the amount of glucose released between 20 and 120 min of digestion. The rate of digestion can be influenced by factors like the branched structure of amylopectin which makes it more susceptible to hydrolysis than amylose \(^{(67)}\), therefore the amylose to amylopectin ratio have a significant effect on glycemia. Available dietary carbohydrates include starch and glycemic carbohydrates such as glucose and maltose. Classifications such as rapidly available glucose and slowly available glucose from sugar and starch can provide further insight into the physiological effects of carbohydrates. These measurements are done by measuring free glucose as well as glucose release after 20 and 120 min incubation at 37 °C in the presence of pancreatic amylase and amyloglucosidase to determine rapidly and slowly digestible starch content as well as the starch digestion index and rapidly available glucose.

Starch can be further characterized into resistant starch (RS), defined as “the starch and starch degradation products that on average resist digestion in the small intestine” \(^{(2)}\). There are four types of RS; RS1 is physically inaccessible starch, most seen in whole grains. RS2 are native starch granules that are not digested because of conformation or structure shielding. RS3 are non-granular starch-derived materials formed during retrogradation, which is due to cooling or storing gelatinized starch,
defined as the irreversible swelling and/or disruption of the starch granules \(^{(62)}\). Finally, RS4 starches are chemically modified starches \(^{(63)}\). In cereal grains and products the RS proportion of starch is relatively small, typically 0-5% of starch \(^{(64)}\). The amount of RS is influenced by factors like the amount of starch present, food processing, and how the food is cooked and stored \(^{(65)}\). The concentration of RS in barley can be increased through extrusion cooking and pelleting of barley products \(^{(51, 66)}\).

### 2.3.4. Fibre

Since the establishment of the fibre hypothesis by Burkitt and Trowell \(^{(68)}\) our understanding of dietary carbohydrates has progressed greatly with a distinctive interest and role of fibre. The most widely used fibre definition is “the nondigestable carbohydrates and lignin that are intrinsic and intact in plants” \(^{(69)}\). In barley, fibre represents the second major constituent of the grain after starch, but unlike starch, fibre is found throughout the kernel. Fibre can be classified into soluble and insoluble forms. The content of total fibre in barley ranges from 11–34% of which 3–20% is soluble dietary fibre mostly in the form of \(\beta\)-glucan \(^{(57, 70, 71)}\).

### 2.3.5. \(\beta\)-glucan

\(\beta\)-glucans are soluble fibres found in many cereal grains, they are large linear polysaccharides of glucose monomers. Specifically, the mixed linkage \((1\rightarrow3, 1\rightarrow4)\)-\(\beta\)-D-glucans, are linear homopolymers of D-glucopyranosyl residues. Barley is considered to be the richest source of \(\beta\)-glucans which account for approximately 75% of the total cell wall polysaccharides in the endosperm cell walls; the rest consists of arabinoxylans, cellulose, glucamannans and proteins \(^{(57)}\). The recent focus and renewed interest in
barley as a human food is largely due to the health benefits attributed to β-glucan. The β-glucan content of barley can range from approximately 2-11%, which is generally higher than oats (2.2–7.8) and wheat (0.4-1.4%) (57). The health benefits associated with consuming β-glucan rich foods include lowering blood glucose, insulin and blood lipids, in particular, serum total and LDL-cholesterol (28). Some of these effects have been shown to depend on the capacity of β-glucan to increase the viscosity (defined as a measure of resistance to flow) of intestinal content, which in turn depends on β-glucan molecular weight (MW) and solubility (28). Wolever et al (72) conducted a double blind parallel design RCT to determine the physiological effectiveness of high-MW oat β-glucan compared to a medium and low-MW oat β-glucan in lowering LDL cholesterol in 345 subjects. Wolever and colleagues showed that when subjects with high LDL cholesterol consumed a breakfast cereal containing 3 g high-MW oat β-glucan cereal/day, LDL was lowered by 5% but the effect was reduced by 50% in subjects consuming a breakfast meal with a low-MW oat β-glucan. However, the relationship between MW and glycemia or the GI has not been fully examined yet. The consequence of differences in chemical composition, processing, and food form on the MW, viscosity, solubility of β-glucan and their interactions have also not been fully explored.

The process of determining the quality of barley and the physiological effectiveness of barley β-glucan on physiological responses requires the determination of the amount of β-glucan. Total β-glucan content is generally determined based on the overall sample. The most common method for β-glucan determination is the AOAC (73). The method is also known as the McCleary and Codd (74), it involves β-glucan dissolution in a buffer where its hydrolysed to oligosaccharides and glucose using the lichenase and
β-glucanase enzymes respectively. Other available methods include the use of dyes such as Calcoflour which is a specific dye for β-glucan.

2.3.6. Physico-chemical Characteristics of β-glucan

The physico-chemical properties of β-glucan in barley have been suggested to have a key role in affecting postprandial responses in humans. The number of parameters that include the following: MW, solubility, viscosity, microstructure, particle size, chain length, and concentration of β-glucan among other parameters. The physico-chemical properties denote an interaction between the physical properties (e.g. Structure and MW) and the chemical properties (viscosity and chain length) and their impact on physiological activity in vivo. Data in the literature indicate a strong correlation and interdependence between these factors (28, 72, 75-77) and glycemia and the GI. In particular, viscosity and MW are thought to have a superior role and they are mutually associated with the physiological effectiveness of β-glucan. Viscosity is defined as the resistant of a solution to flow and MW, a measure of the size and weight of the polysaccharides in β-glucan, is defined as the following:

$$
M_n = \frac{\sum \eta_i M_i}{\sum \eta_i} = \frac{\sum w_i}{\sum w_i / M_i}
$$

$$
M_w = \frac{\sum \eta_i M_i^2}{\sum \eta_i M_i} = \frac{\sum w_i M_i}{\sum w_i}
$$

where $M_i =$ molar mass of the component molecules of kind i

$n_i =$ number-fraction of the component molecules i

$w_i =$ weight-fraction of the component molecules i

(77)
contribute to the overall viscosity in the gut. β-glucan is mainly a soluble fibre but typically only a portion of the total is extracted under physiological conditions. According to Woods (78), the viscosity of β-glucan is dependent on its extractability and/or solubility and the MW distribution which is in turn controlled by structural and microstructural characteristics of the food consumed. In order to study and assess the physiological effectiveness of β-glucan and appreciate its contribution to health, in particular its contribution to the development of viscosity in the gut, requires extraction and isolation of β-glucan from the overall sample. During the extraction process it is imperative to minimize structural changes that could damage other constituents such as the MW. The functionality and health benefits of β-glucan depend greatly on the nature of β-glucan in the food product consumed. Therefore, it is imperative to learn how extractability and solubility of β-glucan are influenced by processing and cooking. Interestingly, most of the data in the literature on how the physico-chemical properties of β-glucan are influenced by processing have been done either in oats or in products containing isolated β-glucan from oats and barley but not on whole grain barley. For example, Beer et al. (77) found that extractability was increased during the baking of muffins made from oat bran but the MW was decreased. In contrast, the authors found that when oats were cooked as porridge both extractability and MW were not affected. Robertson et al (79) showed that biscuits made from flaked barley, milled barley or only the starch of barley when fed either raw or cooked, extractability of β-glucan increased in the cooked biscuits as measured by an in vitro digestion model.

A number of methods exist to measure the physico-chemical characteristics and the values depend strongly on the methods of analysis and extraction making the
comparison between different reports using a single cereal difficult; this is also true for comparison to other cereals. The use of rheological measurements using instruments like rehometers and viscometers are the most frequent method of determining viscosity \(^{(80)}\). This involves the use of *in vitro* digestion models to estimate overall or apparent viscosity of food slurry in order to directly measure β-glucan extract viscosity. This is an important strategy which recognize that the viscosity of gastrointestinal product is perhaps more relevant than sample viscosity in its effect on physiological effects. Methods of MW analysis include various chromatographic techniques with light scattering detections. High-performance size-exclusion chromatography (HPSEC) equipped with post-column dying with Calcoflour, a fluorescent dye that specifically binds to β-glucan \(^{(81)}\), is the most often used method of determining MW \(^{(75)}\).

![Figure 2. Principle of the mixed-linkage β-glucan assay procedure.](image-url)
2.3.7. Barley Processing and Cooking

Preparing barley for human consumption requires processing and cooking, since most barley cultivars are hulled, removing the hulls or dehulling is necessary. The most common method of processing is “Pearling” considered as one of the oldest practices used in the processing of barley. This process of abrasion of the barley kernel involves the successive removal of grain tissue starting with the outer layers of barley and working inward. Dehulling by pearling still renders the barley grain as a whole grain, because the germ, endosperm and bran layers are still intact. Depending on the amount of materials removed, the rate of pearling, which is dictated by cultural preferences, can produce barley cultivars labelled as: wholegrain (only the husk was removed), pot (dehulled and further removal of pericarp) and white-pearled (WP) (all the bran and most of the germ and crease removed) \(^{(27)}\) (Figure 1). Varying the degree of pearling time resulted in significant alterations in the chemical and nutritional composition of barley. These changes include decreasing total fibre but not soluble fibre \(^{(82)}\), increased concentration of starch and β-glucan; this can be done without significant effects on the endosperm \(^{(83)}\) but only up to a pearling degree of 15% \(^{(84)}\). Pearling is considered as a beneficial method of creating barley fraction with specific characteristics (i.e. high/low starch, high β-glucan) for different end uses such as the addition of smaller amount of barley to foods or to incorporate barley as a functional food ingredient. Barley can also be milled using a roller mill to produce barley flour and bran, this is considered an uncommon practice and needs to be further explored \(^{(85)}\). It’s generally assumed that barley bran consists of the testa, pericarp, germ, aleurone and the subaleurone layers; however, since barley is
pearled before milling, the bran and flour composition may differ depending on the degree of pearling \(^{(27)}\). Abrasion milling and sieving is another form of barley milling, which involves the milling of dehulled or hull-less barley by an abrasion mill and sieving the ground material through a series of sieves with an option of different sizes for the openings.

Extrusion cooking is a popular industrial technique for the production of breakfast cereals, breads, pasta and cooked flour \(^{(27)}\). This process employs simultaneous actions of temperature, pressure and shear at differing level of intensities. Other forms of cooking include hydrothermal treatments \(^{(27)}\). These processing methods are thought to aim principally on enhancing the nutritional value of barley and creating longer shelf-life and convenient barley based products. Nonetheless, their impact on the metabolic effect of barley in humans is not known. Further, the effect of these processes on the physico-chemical properties of barley is also unknown.

Processing and cooking cause’s major changes in the architecture of the grain, mostly in the cell wall matrix. Exposing starch rich foods to boiled water can result in significant changes to starch properties. Starch can go through transformations that can affect its digestibility such as gelatinization. Gelatinization occurs when starch is heated in water; it is the disruption of molecular structures within the starch granule. This leads to swelling in the granules due to increased water absorption which coincides with leaching of material from the starch granules, mostly amylose \(^{(61-62)}\). Changes can also occur to the particle size either due to pearling, milling or cooking thereby reducing the particle size. This leads to more exposure per surface area to digestive enzymes and consequently accelerates starch hydrolysis and the digestion and absorption processes.
2.4. Health benefits of barley

The renewed interest in barley comes from its ability to produce favorable effects on a number of disease risk factors such as post-prandial glycemic responses, blood lipids and blood pressure (26). Barley has also been suggested as satiety inducing food due to its low GI values and high viscous fibre content, yet this has not been fully explored. The synergistic effect between barley and the GI by which they induce these favorable effects also needs to be examined. Recently health Canada accepted a health claim linking the consumption of barley β-glucan to reducing blood cholesterol (25). This claim is based on evidence showing that the consumption of at least three grams of β-glucan per day helps lower cholesterol (86).

2.4.1. Barley and blood lipids

Reducing serum LDL-cholesterol concentrations has been shown to reduce the risk of coronary artery disease (CAD) (87). There is good evidence in the literature to suggest that whole grains high in viscous soluble fibre such as oats and barley are more effective in lowering blood lipids than other grains such as wheat or rice (88). The suggested mechanism of cholesterol lowering after consuming a soluble fibre rich diet include delayed intestinal absorption of lipids and inhibition of absorption and reabsorption of cholesterol and bile acids alongside an increased excretion of bile acids (86). These effects are believed to be induced by β-glucan ability to increase the viscosity of the intestinal content (28). Other factors may also be responsible, such as the fermentation of soluble fibre in the colon, resulting in production of short-chain fatty
acids inhibiting cholesterol biosynthesis \(^{(89)}\).

Since the approval of a food health claim of the cholesterol lowering abilities of oat \(\beta\)-glucan by the FDA in 1997, numerous reports of the effects of oats and barley products on blood lipids, mostly with oats, have been published. Interest in barley gained further attention due to its high content of soluble fibre, mostly \(\beta\)-glucan and its very low GI values \((20 – 46)\) \(^{(90)}\). This has been translated into a number of health claims for barley per se \(^{(25, 91)}\). Most of the available data on barley or barley products are related to cholesterol lowering ability with a few investigating other disease end point and metabolic markers such as glycemia, hypertension, inflammation and satiety. More importantly, most of the interventions are done in harshly processed barley, barley enriched food products and extracted \(\beta\)-glucan with a few using intact whole grain barley kernels.

Recently, AbuMweis et al \(^{(86)}\) conducted a meta-analysis to accurately quantify the effect of barley \(\beta\)-glucan on blood lipids concentrations in humans and assess factors that could affect its efficacy. AbuMweis and colleagues identified 11 studies investigating the effect of barley \(\beta\)-glucan on blood lipids, of which 2 studies did not find an effect \(^{(92, 93)}\). Overall, they found a reduction in weighted mean effect size of 0.30 mmol/l \((95\% \text{ CI: } -0.39, \text{ to } -0.21, \text{ P}<0.00001)\) and 0.27 mmol/l \((95\% \text{ CI: } -0.34, \text{ to } -0.20, \text{ P}<0.00001)\) for total and LDL cholesterol compared with control without finding a dose-dependent response. The authors note that the lack of effects in the two studies are due to issues like MW and the use of extracted barley \(\beta\)-glucan in different food products and forms, factors that have not been comprehensively investigated when assessing the effects of barley on glycemia and satiety. They also note the importance of barley
physico-chemical characteristic such as MW and solubility as determinants of barley’s ability to lower cholesterol, an observation that have been reconfirmed recently (72). The authors further conducted a subgroup analysis comparing the interventions that used barley vs. extractable β-glucan from barley, they found that the reduction in total cholesterol was only significant in barley but not the extracted β-glucan products (P<0.00001).

2.4.2. Barley and Glycemia

Global data show an un-abating upward trajectory in diabetes rates with 366 million people suffering from diabetes in 2011-2012 worldwide (94). T2D is characterized by insulin resistance and reduced insulin secretion (95). Therefore, food products that decrease plasma glucose and insulin demands may plausibly reduce the risk of developing T2D (96). Data also shows that increased blood glucose responses produce undesirable consequences on health, an occurrence known as hyperglycemia (97). Post-prandial hyperglycemia is characterized by high blood glucose concentrations post meal, which is a strong predictor for developing T2D. Hyperglycemia and constant fluctuations in blood glucose have been further associated with increasing oxidative stress, protein glycation and inflammatory responses (98), all of which are risk factors for a number of chronic illness that share a common underlying pathophysiological mechanism (96).

Barley and barley food products have been shown to produce favorable effects on glycemia (44). The mechanism responsible for these effects have been suggested to be related to the ability of barley β-glucan in its original state which possess a very high-MW that exhibits high viscosity at a low concentration. Consuming β-glucan rich barley can increase the viscosity of the meal bolus in the stomach reducing the mixing of food
with digestive enzymes and delaying gastric emptying \(^{(99)}\). Increasing the viscosity has also been shown to retard the absorption of glucose \(^{(100)}\) and slow the rate of starch digestion in \textit{in vitro} digestion model studies \(^{(75, 101, 102)}\).

Tosh \(^{(103)}\) conducted a recent comprehensive review of human studies investigating the role of barley and oat food products in lowering post-prandial blood glucose. Of the 34 studies identified by the author to fit the selection criteria, 10 studies used barley products in the intervention and 6 used both barley and oat food products. The treatments included 64 barley products of which only 64\% demonstrated a significant effect in reducing AUC or the GI (GI reduction in a barley enriched products such as bread). The average \(\beta\)-glucan dose in barley products was 4.7 ± 2.5 g with a range of change in AUC − 1.4 to − 147 mmol.min/l (the average AUC change was -54 ± 39 mmol.min/l), a reduction that may be considered clinically relevant. Studies with intact barley kernels are scarce, only two previous studies have used intact barley \(^{(102, 104)}\), and evidence of an association between the naturally occurring \(\beta\)-glucan in intact barley and the presumed health benefits is limited. Most studies in the literature have used barley enriched products or extracted and concentrated barley \(\beta\)-glucan to assess the effects of barley on postprandial responses, an extrapolation that may not be precise. Granfeldt et al \(^{(102)}\) conducted an intervention using boiled intact (rice replacement) and milled (porridge) barley kernels with different amylose – amylopectin ratio to assess the glucose and insulin responses to these products. All the barley kernels and porridge products elicited a lower blood glucose response compared to the control, a white wheat bread (\(P<0.05\)). The intact barley kernels produced a significantly lower response than their corresponding boiled flours (porridge) (\(P<0.05\)) with no differences among the
intact kernels or in the GI among all treatments. The authors also measured the insulin AUC and an insulin index and found no significant differences between barley products including the milled flour.

The Granfeldt et al study has limitation that makes it difficult to interpret; (1) all the barley test foods were consumed as part of a meal which included protein (19g), fat (8.4g) 200 ml of water and 150 ml of coffee or tea. This may dilute the interpretation of the direct observations due to effects like stomach distension, visual perception and feelings of fullness due to increased meal volume and energy content; (2) the barley cultivars tested may not be applicable to cultivars grown in Canada or North America in general. The study also lacks a comprehensive characterisation of starch and the physico-chemical properties of barley cultivars.

As mentioned previously there is strong evidence that the main factor responsible for the low glycemic response to barley foods is related to the viscosity of β-glucan, therefore studies assessing barley impact on postprandial responses should take into consideration such characteristics and examine factors that impact them. This was first revealed by Jenkins et al (105), where they were the first to demonstrate that the capability of certain soluble fibres to lower post-prandial glycemia was lost after acid hydrolysis; the authors also observed a relationship between viscosity and glycemic responses. This work was the prelude to the conception of the glycemic index by the same group.

2.5. THE GLYCEMIC INDEX

2.5.1. Overview

During the 1970s, interest in comparing glycemic responses elicited by different
carbohydrates began to rise leading to indications that post-prandial blood glucose responses are not the same in different CHO rich foods \(^{(3, 105-109)}\). The systematic classification of foods according to their glycemic responses was first undertaken by Otto and Niklas \(^{(110)}\), as an approach to incorporate foods into diabetic diets in quantities inversely proportional to their glycemic responses. In 1981, the glycemic index concept was introduced by Jenkins and his colleagues as a physiological metric classification that provided scientists and clinicians with a physiological ranking of CHO rich food potential to raise blood glucose \(^{(12)}\). The concept was an extension of the Fibre hypothesis of Burkitt and Trowell \(^{(68)}\), which suggests that foods that are slowly absorbed may have metabolic benefits in relation to diabetes and to the reduction of coronary heart disease. Initially, it was thought that GI was only relevant in the treatment of Diabetes \(^{(14)}\). However, the increased interest in research related to the GI has progressed and further clinical utilities have been revealed.

### 2.5.2. Definition

The glycemic index is defined as the area under the blood glucose response curve elicited by a 50 g available carbohydrate portion of a food expressed as a percentage of the response after 50 g anhydrous glucose (reference food) taken by the same subject. The area under the curve (AUC) is calculated using the trapezoid rule. To serve the purpose of the GI the AUC is calculated using the incremental area under the curve ignoring the area below fasting (IAUC) \(^{(110,111)}\). GI is mathematically defined as follows:

\[
GI = \left( \sum_{x=1}^{n} 100 \times \frac{F_x}{\bar{G}_x} \right) / n
\]

\(^{(12)}\)
where $F_x$ is the incremental area under the blood glucose response curve (AUC) in subject $x$ elicited by 50g available carbohydrate from the test food and $\bar{G}_x$ is mean the AUC in subject $x$ elicited by 50g glucose tested on 2 or 3 separate occasions. The GI is the mean of these values in $n$ subjects; the current internationally accepted GI method (13) stipulates that $n \geq 10$. Thus, any so-called GI value not based on equation [cite] with $n \geq 10$ is not a valid GI. Several key implications of the definition of GI as given in equation are (12). GI is a property of foods which is measured in human subjects; GI is not a glycemic response; and GI is a property of high carbohydrate foods tested alone (not as part of a mixed meal).

### 2.5.3. Glycemic Index Methodology

In 1998, GI methodology was discussed as part of a global meeting on the role of dietary carbohydrates in nutrition. The joint committee of FAO/WHO Expert Consultation has agreed on a reference methodology (1), and provided recommendation in regards to the practical application of GI. The recommendation included “the glycemic index can be used, in conjunction with information about food composition, to guide food choices” and “at least 55% of energy be derived from carbohydrates and that the bulk of carbohydrate foods be those rich in dietary fibre and with a low glycemic index”. The methodology has since gone a more detailed review in 2005 (112) and an Australian method was recognized in 2007 (113). These methods were further evaluated in an international inter-laboratory studies in 2003 (114) and 2008 (115) to further enhance the precision and validity of the methods. More recently, an official International Standards
Organization (ISO) method for the GI has been published\textsuperscript{(13)}.

The protocol is recommended to adhere to the following procedures: The recruitment of healthy male or female subjects between the ages of 18-75, for most purposes 10 subjects or more is recommended. Subjects should be studied in morning between the hours 7:00-9:30 am after an overnight fast of 10-14 hours. On each test occasion the subject is weighed and two fasting blood samples are obtained by finger-prick, after that the subjects start to consume the test meal within 10-15 minutes, where a timer is started with the first bite of the test meal. Additional blood samples are obtained at 15, 30, 45, 60, 90, and 120 minutes after starting to eat. The test meals consist of portions of the test food containing 50 g available carbohydrate. Subjects should have a drink with the test meal, and can choose to have one or two cups of water, tea or coffee with or without 2% milk (30 ml) and artificial sweetener. The volume and type of drink the subject choose should remain the same for all tests done by that subject. GI studies are mostly done in a series of tests including a certain number of test foods and at least three tests of the reference food. Reference food can be in the form of anhydrous glucose or white bread which is used most often. The IAUC is calculated using the trapezoid rule. The individual IAUC values for each test food in each subject are expressed as a percentage of the mean IAUC value for the repeated reference food tests taken by the same subject. The mean of the resulting values for each food is the GI value for that food\textsuperscript{(ISO, 2010)}\textsuperscript{(13)}.

2.5.4. Clinical Utility and Implications of Glycemic Index

Originally the intended utility of the GI was to be used in supplement with information about the composition of foods in planning diets for diabetic patients. This is
supported by strong evidence showing that reducing the GI of diet improves glycemic control \(^{15,16}\). A meta-analysis by Brand-Miller et al., which included 14 studies showed that low-GI diets significantly reduced HbA\(_{1c}\) by 0.43\% (the 95\% confidence interval: 0.72 - 0.13) compared to a high-GI diets. This evidence is reinforced by a number of large studies \(^{116-118}\). In the last decade interest in the GI concept has exponentially increased revealing further clinical utilities of the GI, beyond that of managing or reducing the risk of developing diabetes \(^{119}\), and that may be relevant to a wide range of individuals. These include: weight maintenance \(^{120}\) and reducing the risk for CVD \(^{10}\), Stroke \(^{121}\), metabolic syndrome \(^{122}\), inflammation \(^{18}\) and a number of cancers \(^{123,124}\). The GI is most commonly used to rank foods as low-GI (\(\leq 55\)), medium-GI (56-69) or high-GI (\(\geq 70\)) to be utilized in clinical practices guiding the consumption of low-GI foods more often \(^{13}\). Indeed, there is evidence to suggest that low-GI diets have at least as many, if not more, statistically significant health positive effects than there is for whole grains and fibre \(^{13,16}\). Jenkins et al compared the effects of a low-GI diet on glycemic control compared to a high-cereal fibre diet in 210 T2D individuals for 6 months and found that the low-GI diet reduced HbA\(_{1c}\) by \(-0.50\%\) (95\% CI, \(-0.61\%\) to \(-0.39\%\)) compared to the high-cereal fibre diet \(-0.18\%\) (95\% CI, \(-0.29\%\) to \(-0.07\%\)) (\(P < 0.001\)); such reduction is considered clinically meaningful in the management of diabetic individuals. Therefore, it appears that soluble and viscous fibre rich whole grains and the GI share a synergistic effect and complement each other. Taken together, such effects have promoted the interest in whole grains that are low-GI and rich source of viscous fibre. Such low-GI foods have been suggested to hold a higher potential as satiety inducing foods \(^{54,125}\).
2.5.5. Glycemic index and satiety

Satiety is defined as the state in which further eating is inhibited and occurs as a response of having eaten \(^{(126)}\). Low-GI diets have been promoted for weight loss and maintenance due to a number of factors including their positive effects on glycemia and satiety, which consequently may have a role in regulating appetite \(^{(17, 18, 127-130)}\). This is consistent in part with the glucostatic hypothesis postulated by Mayer \(^{(131)}\) 60 years ago. The low-GI satiety hypothesis stipulates that low-GI foods are absorbed slowly resulting in a slowed gastric emptying, which then produces less variation in glycemic responses and in turn could increase satiety and appetite regulation \(^{(128)}\). However, this claim is in contrast to evidence indicating that satiety is no longer influenced by glycemic responses per se over 2 hours after eating \(^{(132)}\). In addition there is further evidence showing early satiating effect of high-GI CHO such as glucose in suppressing short term food intake 60-90 min after the ingestion of a preload compared to a low GI food \(^{(133)}\). Moreover, two recent experimental studies found that low-GI test meals had no effect on satiety or food intake \(^{(134, 135)}\). On the other hand, Low-GI foods are generally also high in fibre, which may play a role in its effects on appetite. In epidemiological studies, fibre have been found to reduce weight gain over time and have favorable effects on glycemia \(^{(136)}\) and in short-term experimental studies fibre has been found to increase satiety and reduce food intake \(^{(137-139)}\), but not all studies found an effect \(^{(126)}\). For example, Mattes tested a combination of functional fibre systems and found no effect of guar and alginate fibres in a solid food matrix on appetite \(^{(140)}\). This observation is thought to be due to the use of extracted fibre and/or use of synthetic fibre.

Literature in this area is still inconclusive and warrants further research. As
mentioned previously not all fibres are equal and the importance of viscosity seems to be a crucial factor \(^{(21)}\). This in turn depends on the fibres inherent physico-chemical properties and whether they differ between different sources of fibre. Similarly, the ability of fibre to maintain the physico-chemical capacity during processing is equally important. These factors have led to interest in finding foods with robust physico-chemical properties and as an ample source of \(\beta\)-glucan soluble fibre. In North America and Europe, cereal grains are generally the main source of fibre in the diet. Barley, a low-GI whole grain holds great potential as an alternative to current grains and as a functional food product due to its high \(\beta\)-glucan fibre content and very low GI values, which are crucial factors when promoting the consumption of whole grains. Many new barley cultivar varieties have been specifically produced for human consumption with unique functional characteristics in response to the favorable effects of \(\beta\)-glucan on health and the 2006 FDA and the 2012 Health Canada approval of claims using \(\beta\)-glucan and barley products. Yet, whether factors like compositional differences in barley cultivars, level of pearling and food form influence post prandial responses of barley and consequently affect their capacity to induce satiety is not known.

Nonetheless, studies examining the effect of barley kernels per se on satiety are scarce and their interpretation is difficult. Most studies have examined barley in the form of enriched food products rather than whole grain kernels or as a 100% barley based products. Moreover, barley studies are further complicated by differences in chemical composition and food preparation methods. For example, Granfeldt et al \(^{(102)}\) fed four different barley genotypes with different amylose-amylopectin ratios (7 - 44% amylose) as whole kernels and milled flour (porridge) and compared their effects on satiety to
white bread. All barleys had a significantly higher satiety area except the normal barley kernels and their corresponding flour compared to white bread; these results may be due to the higher RS content in the more satiety inducing test meals (2.00-3.00 g/serving) compared to the other products (0.18-0.8 g/serving) with the flour products containing higher RS content compared to their corresponding kernel products. The high RS content may be due to the retrogradation of starch in response to heat treatment, mainly amylose. Similar results and explanation apply to the study by Liljeberg et al and Nilsson et al (141, 142). Kaplan and Greenwood (143) also looked at the effects of barley on satiety compared to a placebo drink, Glucose drink and instant mashed potatoes in an elderly population. Compared to the placebo and the glucose drinks barley and mashed potatoes elicited a higher satiety scores (P <0.008), with the mashed potatoes increasing satiety significantly more than Barley (P = 0.015) despite it being a high GI food. Moreover, Barley in this study was consumed with 2.5 g of butter which may have led to higher satiety compared to placebo and to the glucose drinks due to a slower gastric emptying by virtue of fat. The reference food in this study may also be an issue as liquids have been shown to be less satiating than solid foods (144). Finally in this study the author’s also observed that by 120 min post ingestion, barley no longer produced higher satiety than glucose and placebo. Recently, the EFSA rejected a satiety food claim for barley and oat products in Europe on the basis of lack of substantial evidence, the report submitted to the EFSA included two studies that assessed subjective satiety and only did so on a single occasion (145). Health Canada has recently been engaged in drafting a document to allow and regulate satiety food claims for products sold Canadian markets (146). This reflects the increased interest in barley.
The increased interest in barley stems from 2 major reasons: (1) its demonstrated health effects; (2) potential as replacement for many stable refined grains found in our diets such as white bread and rice. The establishment of government regulated health claims for barley reflects its heightened consumer demand. Despite the demonstrated health effects of barley we still lack an understanding of the effects of chemical differences and processing methods on the health benefits of barley. Factors such as the compositional diversity in barley cultivars, solubility, viscosity, molecular weight (MW), food form and cooking may influence the post prandial responses of barley products, alter their GI and subsequently affect subjective satiety. There are gaps in our knowledge with respect to the contributions of the physico-chemical properties to the positive effects of whole grains on metabolic risk factors such as glycemia. Questions remain unanswered regarding the contribution of the GI, fibre, physical structure to the positive effects of barley and whether these effects are modified by any of the aforementioned factors.

In the next chapter, chapter 2, I will present my work examining the contribution of chemical composition including starch composition and its nature (slowly digested starch vs. rapidly digested starch), total fibre and β-glucan content, pearling and food form on glycemia and the GI of different barley cultivars.
3. Research Questions

This thesis will attempt to answer the following questions:

5. What is the significance of differences in chemical composition, total fibre and β-glucan content on glycemic response, GI and subjective satiety?

6. What is the effect of food form on glycemic response, GI and subjective satiety?

7. What is the magnitude and impact of processing and food form on the physico-chemical properties of barley; is there a synergistic effect of the physico-chemical properties?

8. What is the contribution of the physico-chemical properties with respect to glycemia and the GI?

3.1 Objectives

• Primary Objective:
  • Examine the effects of differences in barley cultivars and chemical composition on glycemic response, GI and subjective satiety
  • Examine the effects of differences in level of pearling on glycemic response, GI and subjective satiety
  • Examine the effects of changing the food form on glycemic response, GI and subjective satiety

Chapters 4 and 5 will represent the three in vivo randomized clinical trials (RCT) addressing the first three previously mentioned questions. Chapter 4 will assess the contribution of chemical composition including starch composition and its nature (slowly
digested starch vs. rapidly digested starch), total fibre and β-glucan content, pearling and food form on glycemia and the GI of barley. Chapter 5 will address the effects of differences in chemical composition, pearling and food form on subjective satiety and the satiety index score (SI). Chapter 6 will investigate the contribution of the physico-chemical properties on glycemia and the GI; in particular, the impact of molecular weight, viscosity, solubility and the bioactivity of β-glucan using an in vitro digestive model. Chapter 7 will explore the significance and implications of this work and synthesise chapters 4, 5 and 6. This will help identify determinates of the quality of barley and barley food products and their impact on physiological health outcomes. Ultimately, our intention is to use barley as a surrogate measure of how we classify whole grains.
4. Barley Cultivar, Kernel Composition and Processing Affects the Glycemic Index

The following Chapter is a reproduction of a manuscript that has been published in the

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4.1. Abstract

Barley has a low Glycemic Index (GI), but it is unknown whether its GI is affected by variation in carbohydrate composition in different cultivars and by food-processing and food-form. To examine the effect of these factors on GI, 9 barley cultivars varying in amylose and β-glucan content, were studied in 3 experiments in separate groups of 10 healthy participants. Experiment 1: three barley cultivars underwent 2 levels of processing: hull removal (wholegrain, WG) and bran, germ, and crease removal (white-pearled, WP). GI varied by cultivar (CDC-Fibar, 26 ± 3 vs. AC-Parkhill, 35 ± 4, \( P < 0.05 \)) and pearling (WG, 26 ± 4 vs. WP 35 ± 3, \( P < 0.05 \)) with no cultivar×pearling interaction. Experiment 2: the GI of 7 WG cultivars ranged from 21 ± 4 to 36 ± 8 (\( P = 0.09 \)). Experiment 3: WG and WP AC-Parkhill and Celebrity cultivars were ground and made into wet pasta. The GI of AC-Parkhill pasta, 69 ± 3, was similar to that of Celebrity pasta, 64 ± 4, but unlike Experiment 1, the GI of WP pasta, 61 ± 3, was less than WG pasta, 72 ± 4 (\( P < 0.05 \)). Pooled data from experiments 1 and 2 showed that GI was correlated with total fiber (\( r = -0.75, \ P = 0.002 \)) but not with measures of starch characteristics. We conclude that the GI of barley is influenced by cultivar, processing and food form, but is not predicted by its content of amylose or other starch characteristics.
4.2. Introduction

The growing availability and intake of refined carbohydrates especially those with high glycemic index (GI) combined with rise in the obesity epidemic have contributed to a rise in cardio-metabolic disorders \(^{10}\). The latest reports from international studies show an unabating upward trajectory in diabetes rates with 366 million people suffering from diabetes in 2011 worldwide \(^{94}\). Type 2 diabetes is characterized by insulin resistance and reduced insulin secretion \(^{119, 147}\). Therefore, food products that decrease plasma glucose and insulin demands plausibly reduce the risk of type 2 diabetes \(^{148}\). This has renewed interest in wholegrain cereals with intact botanical structure that are slowly digested and have a low-GI. The GI introduced 30 years ago, is a physiological indicator that ranks carbohydrate rich foods based on their potential to raise blood glucose. By replacing high-GI refined carbohydrates such as white rice or white bread with low-GI wholegrain products such as barley, consumers can meet nutrition recommendations to consume wholegrain foods daily and reduce diet GI, maneuvers which may slow the progression of chronic diseases. However, the consumption of barley in North America is very low, at least in part due to lack of availability of barley food products which are convenient to purchase and prepare. Recently, many new barley cultivars have been specifically produced for food use with unique functional characteristics that improve their versatility for use as food products with enhanced health benefits such as high β-glucan and slowly digested starch (SDS) barley. The genetic and compositional diversity in barley cultivars and other factors such as the level of pearling, amylose-to-amylopectin ratio, food form and cooking may influence post prandial responses of barley products and alter their GI. It has been suggested that GI values do not necessarily indicate the
rate and extent of carbohydrate absorption, but rather are determined by the combined effect of all the properties of the grain which influence the rate of entry and removal of glucose from the blood stream\textsuperscript{(149)}. Elucidation of the role of carbohydrate quality in health promotion requires a better understanding of how the physico-chemical characteristics of food and processing methods such as pearling, a common commercial process whereby the husk and outer layers of barley grains are removed by a friction and abrasion process, relate to their physiological responses. This will also provide additional insight to the concept of GI. Therefore, the objective of this study was to examine the effects of pearling, barley cultivar, physico-chemical properties and food form on the GI of 9 barley cultivars in healthy participants. This project was completed in 3 separate experiments, the purpose of study 1 and 2 was to examine the effects of barley cultivar and level of pearling (e.g. wholegrain, commercial pearled, pot pearled and white pearled). In study 3 a novel in-house wet pasta-like product was manufactured from 100% barley flour to assess the effect of food form on the glycemic index.

4.3. Materials and Methods

All experiments were performed using protocols approved by the Research Ethics Board at the University of Toronto. All participants provided written informed consent to participate. A total of 3 experiments, each with a randomized design were performed in separate groups of 10 healthy participants in the morning after a 10-12 h overnight fast.

Barley Samples

Nine Canadian barley cultivars were selected based on diversity in starch, amylose and β-glucan content and also agronomic purposes. The selected barley cultivars
included 3 two-row, hulled, normal barleys (AC parkhill, Chief, GB 992027); 3 six-rowed, hulled, normal barleys (AC Klink, Celebrity, OAC Kawatha); 1 two-row hull-less, normal barley (AC Alberta); and 2 two-row, hull-less, waxy barley (CDC Fibar, CDC Rattan). Kernels from two-row barleys are generally larger and more uniform in size than those from six-rowed barleys due to crowding of spikelet’s on the spike in the latter. Hull-less barley are free-threshing or naked grains. Normal barley contains starch with an amylose-to-amylopectin ratio of ≈ 1:3, whereas the starch in waxy barleys is almost entirely amylopectin. Characteristics of a majority of the selected barley cultivars and their grains were reported previously \(^{150, 151}\). All cultivars were from Canadian suppliers, the majority obtained from Cribit Seeds (West Montrose, ON, Canada). The waxy cultivars were supplied by the University of Saskatchewan (Saskatoon, SK, Canada). Samples were provided in 25 kg amounts and a 3 kg representative sample of each cultivar was obtained from the original bags for this study. Barley kernels were pearled in 100g batches for various times to achieve the desired level using an abrasive mill (model TM05, Satake, Tokyo, Japan) \(^{82}\). Only barley fractions used in the current study are presented in Table 4, the entire fractions were described in a previous study \(^{82}\).

**Carbohydrate Analysis**

The results of carbohydrate analysis have been reported previously \(^{82}\) but are included briefly here for comparison with *in vivo* glycemic responses. Barley wholegrain and pearled fractions were ground for chemical analysis using a cyclone sample mill (Udy, Fort Collins, CO) equipped with a 0.5-mm screen and stored at 4°C prior to analysis. Moisture and total starch were determined using AACC Approved Methods 44-
Glucose was measured using glucose oxidase/peroxidase reagent and Carey 3C UV-visible spectrophotometer (Varian Techtron, Australia). Amylose and amylopectin were measured using a commercially available method (Megazyme, Wicklow, Ireland). Rapidly digestible starch (RDS), slowly digestible starch (SDS), and rapidly available glucose (RAG) were determined according to Englyst et al. Method (2). Resistant starch (RS) was determined using AACC Approved Method 32-40 and total, soluble, and insoluble dietary fiber using an enzymatic gravimetric procedure AACC Approved Method 32-07 (152). The barley test meals were fed in portions containing 50g available carbohydrate, which was defined as available starch plus free sugars.

**Experiment 1**

The participants in experiment 1 were 6 women and 4 men aged 40.6 ± 2.7 y, with BMI, 27.6 ± 1.2 kg/m². The objective was to determine the effect on GI of two extreme levels of pearling in 3 cultivars of barley varying in amylose and β-glucan content. The cultivars chosen for this experiment were AC Parkhill (high amylose, low β-glucan), Celebrity (high amylose, medium β-glucan) and CDC Fibar (low amylose, high β-glucan) (Table 1). The levels of pearling included wholegrain (WG) (only the husk was removed) and white-pearled (WP) (all the bran and most of the germ and crease removed).

Participants came to the laboratory between 07:30 and 09:45h on 9 separate occasions after 10-12h overnight fasts. On each occasion, after being weighed and giving a fasting blood sample, participants consumed a test meal containing 50g available carbohydrate within 15min and further finger-prick blood samples were obtained at 15,
30, 45, 60, 90 and 120min after starting to eat. Participants chose to drink with the test meal a cup of water, tea, or coffee with 30 mL of 2% milk and/or artificial sweetener if desired. Type of drink chosen by subject stayed constant during the whole study. During the course of the 2h test period, participants remained seated. Six of the test meals consumed by each subject consisted of the 3 different cultivars of barley each pearled to 2 different levels (WG or WP); these were cooked as described below and fed in randomized order. Participants also tested the reference food, white bread, 3 times (1st, 5th and 9th tests). White bread was baked in an automatic bread maker as previously described (153).

**Experiment 2**

The participants in experiment 2 were 6 women and 4 men aged 46.6 ± 4.4 y with BMI 26.4 ± 1.1 kg/m². The objective was to examine the effect on GI of 6 additional barley cultivars and of two additional intermediate pearling levels. Seven barley cultivars were included (Tables 1 and 2); 6 were studied only in the WG state, while 1 cultivar (Celebrity) was pearled to reproduce four fractions: WG, commercial pearled (CP) (some bran and germ were also removed), pot pearled (PP) (all bran and most of the germ removed) and WP. Participants were, therefore, studied on 13 occasions; they consumed 10 different barley test meals in randomized order and white bread 3 times using the same procedures as described for experiment 1.

**Experiment 3**

The participants in experiment 3 were 8 women and 2 men aged 40.5 ± 4.7y with BMI 28.3 ± 2.0 kg/m². The objective was to determine the effect on GI of making pasta from barley flour, using barley cultivars shown in experiments 1 and 2 to have different
GI values (AC Parkhill and Celebrity) each pearled to different levels (WG and WP). Barley samples were milled into flour using an analytical mill (A-10, Tekmar, Cincinnati, OH) and made into a wet pasta-like product using 100% barley flour from each cultivar. Barley flour (100 g) was mixed with water (60-70 mL) to form a paste which was extruded using a pasta maker (PastaMatic® MX700, Treviso, Italy). As a control, wet durum semolina pasta (100 g) mixed with water (35 mL) was manufactured under the same conditions using commercial durum semolina (Robin Hood®, Cargill, Incorporated, Minneapolis, MN). All products were analyzed for dry matter (DM) and wet and dry loss. Salt (1g), xanthan (1g) and 85µL of annato solution (2.8%) were added per 100g barley flour to improve flavor, texture and color, respectively, and overall appearance. After extruding the pasta, products were refrigerated overnight at 4 C° before being cooked on the next morning.

Cooking Procedures

Based on the in vitro carbohydrate analysis, samples of each barley cultivar were cooked in portions that provide 50 g available carbohydrate (Supporting Materials, Table 5). Single portions of barley grains were rinsed with cold water, added to boiling salted water (1:5 ratio barley: water, w/v), covered and boiled for 25 min (WG and CP fractions) or 30 min (PP and WP fractions), and allowed to sit at room temperature for 10 min prior to being served to the participants. Pasta was cooked in boiling water for 5 min for the barley products and 20 min for the semolina product to obtain aldente (firm to the bite) texture. A ratio of 1:8 pasta: water, w/v, was used to minimize losses of nutrients due to cooking in large amount of water.
Blood Glucose Analysis

Capillary blood samples were collected into flat-bottomed 5-mL plastic tubes with a push cap containing a small amount of sodium fluoride and potassium oxalate as an anticoagulant and preservative and stored at -20ºC prior to analysis of whole blood glucose using an automatic analyzer (Model 2300 STAT, Yellow Springs Instruments, WI).

Statistical Analysis

Results are expressed as means ±SEM. Statistical analysis was done using SAS version 9.3. Incremental areas under the curve (iAUC), ignoring area below the baseline, were calculated using the trapezoid rule as previously described (14). The iAUC for each test meal was expressed as a percentage of the same subject's mean iAUC after white bread, and the resulting values multiplied by 0.71 to convert to the glucose scale (i.e. the GI of glucose = 100). The mean of these values was the GI of the food. Because experiments 1 and 3 had a partly balanced factorial design with 2 factors for the barley test meals (cultivar and level of pearling) but not for the control (white bread), the data from experiments 1 and 2 were analyzed in 2 ways. To determine the significance of the differences in glycemic response between the barley meals and white bread the GI and iAUC values for all the test meals (including white bread) were analyzed using repeated measures analysis of variance (ANOVA). To determine the effects of cultivar and pearling in experiments 1 and 3 the mean iAUC and GI values of the barley test meals (not including white bread controls) were subjected to repeated measures ANOVA to determine the main effects of cultivar and level of pearling and the cultivar × pearling interaction. Similarly, blood glucose concentrations at each time point were subjected to
repeated measures ANOVA in experiments 1 and 3. Since experiment 2 did not have a factorial design, the iAUC and GI values for all test meals were analyzed using repeated measures ANOVA. In all cases, after demonstration of significant heterogeneity by ANOVA, differences between individual means was assessed using Tukey's test to adjust for multiple comparisons. In experiment 1, one subject missed one test meal (Celebrity WG) and the missing result was imputed using a procedure described by Snedecor and Cochrane \(^{(154)}\); to account for the imputed value in the statistical analysis, the error degrees of freedom was reduced by 1. Pearson correlation analysis was used to identify relationships between chemical composition and GI values of the barley products in experiments 1 and 2 combined. The criterion used for statistical significance was 2-tailed \(P < 0.05\).

4.4. Results

Experiment 1

All 6 barley test meals elicited significantly lower glycemic responses than white bread \((P < 0.001)\) (Table 1 and Fig.1). When only the barley test meals were included in the statistical analysis, there was a significant main effect of cultivar \((P = 0.008)\) for iAUC with the response of CDC Fibar being significantly less than that of AC-Parkhill. Pearling tended to increase mean iAUC \((P = 0.07)\). However, there were main effects of both cultivar \((P = 0.013)\) and pearling \((P = 0.046)\) on GI; the GI of CDC Fibar was significantly less than that of AC-Parkhill by 9 GI units and pearling (WP) increased GI by 9 units compared to WG. There was no significant cultivar×pearling interaction for either iAUC \((P = 0.89)\) or GI \((P = 0.87)\).

Experiment 2
All barley test meals elicited significantly lower glycemic responses than white bread. The mean GI values of the barley cultivars processed as WG ranged from 21 to 36 but the differences among barley cultivars were not statistically significant (Table 2). When the celebrity cultivar was subjected to progressively greater degrees of pearling, from WG to CP, PP and WP, mean GI values were $21 \pm 4$, $25 \pm 3$, $22 \pm 3$ and $32 \pm 6$ ($P = 0.09$).

**Experiment 3**

The glycemic responses elicited by the barley pastas did not differ significantly from those elicited by semolina pasta or white bread, and the response elicited by semolina pasta was similar to that after white bread (Table 3 and Fig.2). When only the barley test meals were included in the statistical analysis, there was no significant main effect of cultivar for iAUC ($P = 0.31$) or GI ($P = 0.29$). In contrast to the results from Experiment 1, pearling significantly decreased both the AUC ($P = 0.016$) and GI ($P = 0.029$) of barley pasta. There was no significant cultivar×pearling interaction for either iAUC ($P = 0.80$) or GI ($P = 0.81$).

**Correlation between GI values and barley composition**

There was no significant correlation between the GI values of the barley kernels tested in experiments 1 and 2 and either the amylose, RAG, RDS or RS contents of the test meals (Fig.1). These correlations were not significant whether the results for pearled barley were included or not. The relationship between GI and SDS was not significant when only the WG test meals were included ($r = -0.38$, $n = 9$, $P = 0.32$), but approached
significance when the results for pearled barley were included (Fig.1). The only nutrient in barley to correlate significantly with GI was total fiber, and the correlation was significant whether the WP, PP and CP test meals were included (Figure 1) or not ($r = -0.81$, $n = 9$, $P = 0.008$). The correlation between GI and β-glucan ($r = -0.44$, $n = 14$, $P = 0.11$) was not as good as that between GI and total fiber ($r = -0.75$, $n = 14$, $P = 0.002$).

4.5. Discussion

The results of these experiments suggest that processing, cultivar, chemical properties and food form are all factors that impact the GI of barley. All of the 9 barley cultivars and fractions were low GI (GI range: 21-41) (Tables 1 and 2). This is similar to values reported previously (90, 155). In experiment 1 pearling resulted in a higher GI in all 3 of the cultivars tested. In addition, the cultivars differed in GI with CDC Fibar having a lower GI than AC-Parkhill. This is of interest because of all the barley cultivars tested CDC Fibar contained the lowest amount of amylose and highest amount of β-glucan.

Amylose (unbranched $\alpha$-(1-4) linked molecules) and amylopectin (branched $\alpha$–(1-4) and $\alpha$–(1-6) linkages) are the two chief components of starch. Generally, normal barley starch has a 3:1 ratio of amylopectin to amylose; while waxy starch consists almost entirely of amylopectin. The normal barley cultivars examined in the study had amylopectin: amylose ratio of 3.3:1-3.9:1 and starches in the waxy cultivars, CDC Fibar and CDC rattan were composed of about 96-100% amylopectin. The branched structure of amylopectin is more susceptible to hydrolysis than the nearly linear structure of amylose which increases the rate of digestion (61). The amylose-to-amylopectin ratio influences the rate of starch digestion and in turn dictates the concentration of RDS and
SDS \(^\text{\textsuperscript{156}}\). Thus, it would be predicted that a barley cultivar with a lower percentage of amylose would have a higher GI but this was not the case with CDC Fibar which had the lowest content of amylose but the second-lowest GI of all the cultivars tested here. It could be speculated that the reason why CDC Fibar had a low GI, despite its low amylose content, is because of its high content of β-glucan (soluble fiber) and dietary fiber. β-glucan extracted from oats \(^\text{\textsuperscript{157, 158}}\) and barley \(^\text{\textsuperscript{159, 160}}\) has been shown to reduce glycemic responses when incorporated into foods. However, the overall evidence from this study does not support a strong role of β-glucan in determining the GI of whole and pearled barley kernels because GI was more strongly related to the total fiber content of barley rather than β-glucan content. The results also suggest that the GI of barley is influenced by several competing factors including starch and dietary fiber nutritional fractions and their interactions.

To further elucidate what influenced the GI of barley, we assessed starch digestibility in vitro. Some starches release glucose into the blood stream faster than others. RDS is broken down to glucose in 20 min or less while SDS is digested in 20-120 min \(^\text{\textsuperscript{2}}\). RDS content varied between cultivars with CDC Fibar and CDC Rattan containing significantly higher amounts of RDS (26.9-33.4%) compared to other barleys (14.9-26.2%). Englyst and others have suggested that the GI of cereal products can be explained by their content of slowly available glucose (SAG) and rapidly available glucose (RAG), with RAG being positively correlated with the GI of different cereals \((r = 0.74, P < 0.01)\) \(^\text{\textsuperscript{149}}\). However, in our study RAG did not correlate with the GI. This suggests that the GI of foods cannot necessarily be predicted using \textit{in vitro} methods \(^\text{\textsuperscript{161}}\).
In barley the major form of RS is resistant starch type 1 (RS1), physically inaccessible starch \(^{(66)}\). Previous studies have shown a negative correlation between RS and the GI of barley \(^{(162)}\). However, in this study the RS content of all barley cultivars was low, ranging from 0.11-0.49 g/serving. In addition, degree of pearling had a little impact on RS content. On the other hand, total fiber was negatively correlated with the GI of the barley cultivars \(r = -0.81, n = 9, P = 0.008\). Being high in dietary fiber is not necessarily an essential prerequisite of a low-GI food; many common cereals with naturally occurring levels of viscous fiber have a minimal impact on glycemia \(^{(102)}\). Instead, dietary fiber as part of an intact botanical structure, as in barley may be more significant. In this context it is of interest that pearling reduced the total fiber content of barley to a greater extent than that of the \(\beta\)-glucan. This suggests that the starch in the outer layers of barley kernels which are removed by pearling is protected by fiber (not \(\beta\)-glucan) to a greater extent and has a lower GI than the starch in the center of the barley kernels. This might explain why white pearling, which removes 25-30\% of the starch from barley, increases the GI of barley.

In the current study RS was directly measured in barley products, and thus its content is relatively small, and the summation of RDS, SDS and RS contents are lower than total starch content (Supporting materials, Table 5). This discrepancy is due to differences of how starch components were measured; a common practice among researchers is to estimate RS by difference which is not as accurate as the direct measurement of RS used in the current study.

The results of experiments 1 suggest that the matrix structure of barley is a more important determinant of GI than its content of RDS, SDS, RS or \(\beta\)-glucan. This was
also shown by the results of experiment 3 in which disruption of the matrix structure of barley by milling of wholegrain barley greatly increased the GI, despite the pasta containing the same chemical constituents as the wholegrain barley. Our barley pastas had unexpectedly relatively high GI values since pasta is considered to have a low GI. However, we believe our pasta had a high GI because we produced wet or fresh pastas, whereas pasta is usually available as dry pasta. The drying process could harden the matrix structure and make it less accessible to enzymes, and also inactivate indigenous β-glucan degrading enzymes, while wet pasta is more readily available or accessible to indigenous and external enzymes. This was supported by the high GI of the control wet pasta made from semolina flour compared with dry semolina pasta (GI ≈ 41)\(^{(90)}\). It is of interest that pasta made from pearled barley had a lower GI than wholegrain barley pasta, whereas pearling increased the GI of intact barley kernels. We speculate that this may be because there were more particles of insoluble fiber in the wholegrain pasta which gave it a weaker structure and thus more readily able to break apart and be digested.

In the latest 2010 U.S. dietary Guidelines eating wholegrain foods was encouraged. Although we agree with this advice because whole grains contain high levels of important nutrients such as dietary fiber and magnesium, the results of this study are consistent with those of previous studies which suggest that whole grains, that can be processed into a variety of different forms, do not necessarily have a low GI. Therefore, there is a need for improved methods of how whole grains are classified. Selecting a low GI barley cultivar can help not only blunt high postprandial blood glucose levels but also reduce the overall glycemic load of a meal, a dietary maneuver that could produce enhanced public health benefits.
We did not measure insulin responses in this study because the added expense would have limited the number of different cultivars and processing methods we could have tested. In addition, previous studies have shown that barley food products with a low GI also elicit low insulin responses (102). Although we only studied normal subjects, the values may apply to other populations because previous studies suggest that the GI values of starchy foods are similar in normal, hyperinsulinemic and diabetic subjects (163).

In conclusion this study showed that barley cultivar, chemical composition, processing and food form are all significant factors that influence the physico-chemical characteristic of barley and in turn alter the GI. The chemical composition and processing appear to have the biggest impact on the quality of the carbohydrates which may be a determining factor of variations in the GI values of cereal products.
**TABLE 1.** Incremental areas under the curve (iAUC) and glycemic index (GI) of 3 barley cultivars processed (pearled) in 2 different ways, Expt. 1

<table>
<thead>
<tr>
<th>Barley Cultivar</th>
<th>iAUC (mmol×min/L) Fraction</th>
<th>GI</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WG</td>
<td>WP</td>
<td>Fraction</td>
<td>Mean</td>
</tr>
<tr>
<td>Celebrity</td>
<td>71 ± 20</td>
<td>88 ± 14</td>
<td>79 ± 16&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>AC-Parkhill</td>
<td>85 ± 21</td>
<td>109 ± 19</td>
<td>97 ± 19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>61 ± 16</td>
<td>78 ± 11</td>
<td>69 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>Mean of Cultivars</td>
<td>72 ± 18</td>
<td>91 ± 14*</td>
<td>71 ± 14*</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>White Bread</td>
<td>189 ± 22&lt;sup&gt;§&lt;/sup&gt;</td>
<td>71&lt;sup&gt;§&lt;/sup&gt;</td>
<td>71&lt;sup&gt;§&lt;/sup&gt;</td>
<td>71&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SEM for n =10.
<sup>a,b</sup> Significant main effect of cultivar; means with different letter superscripts differ significantly, P < 0.05.
<sup>*</sup> Significant main effect of pearling level, P < 0.05.
<sup>§</sup> White bread differed from all barley test meals, P < 0.05.

There was no significant cultivar×pearling interaction for iAUC or GI.
WG: wholegrain; WP: white pearled.
### TABLE 2 Incremental areas under the curve (iAUC) and glycemic index (GI) of 7 barley cultivars, Expt. 2

<table>
<thead>
<tr>
<th>Barley Cultivar</th>
<th>iAUC (mmol×min/L)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celebrity</td>
<td>48 ± 9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chief</td>
<td>65 ± 11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rattan</td>
<td>58 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26 ± 6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AC Klinck</td>
<td>71 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36 ± 8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kawartha</td>
<td>61 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28 ± 4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AC Alberta</td>
<td>63 ± 13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29 ± 7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GB</td>
<td>52 ± 10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Bread</td>
<td>163 ± 15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values are means ± SEM for n = 10.<br>
<sup>a,b</sup> Means with different letter superscripts differ significantly, P < 0.05.<br>
<sup>§</sup> Cultivars processed to remove the husk only (wholegrain; WG).
TABLE 3 Incremental areas under the curve (iAUC) and glycemic index (GI) of two fractions of pearled barley pasta and semolina pasta, Expt. 3:\$^

<table>
<thead>
<tr>
<th>Barley Cultivar</th>
<th>Fraction</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WG</td>
<td>WP</td>
<td></td>
</tr>
<tr>
<td>Celebrity pasta</td>
<td>137 ± 17</td>
<td>115 ± 17</td>
</tr>
<tr>
<td>AC-Parkhill pasta</td>
<td>141 ± 17</td>
<td>127 ± 12</td>
</tr>
<tr>
<td>Mean of Cultivars</td>
<td>139 ± 14</td>
<td>121 ± 13*</td>
</tr>
<tr>
<td>Semolina pasta</td>
<td>151 ± 20</td>
<td></td>
</tr>
<tr>
<td>White Bread</td>
<td>142 ± 16</td>
<td>71</td>
</tr>
</tbody>
</table>

Values are means ± SEM for \( n = 10 \).
* Significant main effect of pearling level, \( P < 0.05 \).
There was no significant main effect of cultivar and no significant cultivar\(\times\)pearling interaction for iAUC or GI.
WG: wholegrain; WP: white pearled.
\$ Pasta made from 2 barley cultivars pearled in 2 different ways and semolina pasta as a control.
Figure 1: Correlations between the GI and amylose (A), RAG (B), RDS (C), SDS (D), RS (E), and total fiber (F) in WG and pearled barley consumed by \( n = 10 \) (Expt. 1) or \( n = 10 \) (Expt. 2) healthy participants. Filled circles, whole-grain barley; open circles, pearled barley.
Figure 2: GI values of WG and WP fractions of each cultivar and Pasta consumed by $n = 10$ (Expt. 1) or $n = 10$ (Expt. 3) healthy participants ($P < 0.05$). Values are Means±SEM.
Table 4. Percentage of Hulls and Bran Removal (% wb) and Required Pearling Time (sec) of Barley Cultivars

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fraction*</th>
<th>Hull/Bran Removal (%)</th>
<th>Pearling Time (sec)</th>
<th>Moisture (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-Parkhill</td>
<td>WG&lt;sup&gt;1,3&lt;/sup&gt;</td>
<td>11</td>
<td>55</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>WP&lt;sup&gt;1,3&lt;/sup&gt;</td>
<td>31</td>
<td>300</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>WG&lt;sup&gt;1,2,3&lt;/sup&gt;</td>
<td>12</td>
<td>55</td>
<td>10.6</td>
</tr>
<tr>
<td>Celebrity</td>
<td>CP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>17</td>
<td>120</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>PP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>22</td>
<td>195</td>
<td>11.8</td>
</tr>
<tr>
<td></td>
<td>WP&lt;sup&gt;1,2,3&lt;/sup&gt;</td>
<td>32</td>
<td>350</td>
<td>11.8</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WG&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>9.1</td>
</tr>
<tr>
<td></td>
<td>WP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25</td>
<td>370</td>
<td>9.1</td>
</tr>
<tr>
<td>Chief</td>
<td>WG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12</td>
<td>60</td>
<td>12.4</td>
</tr>
<tr>
<td>CDC Rattan</td>
<td>WG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>10.4</td>
</tr>
<tr>
<td>AC Klinck</td>
<td>WG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>11</td>
<td>55</td>
<td>11.9</td>
</tr>
<tr>
<td>Kawartha</td>
<td>WG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12</td>
<td>55</td>
<td>11.5</td>
</tr>
<tr>
<td>Alberta</td>
<td>WG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>11.2</td>
</tr>
<tr>
<td>GB</td>
<td>WG&lt;sup&gt;2&lt;/sup&gt;</td>
<td>12</td>
<td>55</td>
<td>10.7</td>
</tr>
</tbody>
</table>

WG = whole grain, CP = commercial pearled, PP = pot pearled and WP = white pearled. Experiments in which the fractions were tested, n = 3.
## Supporting Material

**Table 5.** Average values for Starch characteristics of barley cultivars and fractions (g/serving)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fraction*</th>
<th>Serving (g)</th>
<th>Total Starch (g)</th>
<th>Amylose (g)</th>
<th>Total Fiber (g)</th>
<th>β-glucan (g)</th>
<th>RAG (g)</th>
<th>RDS (g)</th>
<th>SDS (g)</th>
<th>RS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-Parkhill</td>
<td>WG^{1,3}</td>
<td>87.6</td>
<td>49.3</td>
<td>11.8</td>
<td>16.0</td>
<td>3.8</td>
<td>18.6</td>
<td>14.4</td>
<td>19.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>Wp^{1,3}</td>
<td>76.1</td>
<td>49.7</td>
<td>12.4</td>
<td>10.0</td>
<td>3.4</td>
<td>17.5</td>
<td>15.0</td>
<td>19.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Celebrity</td>
<td>WG^{1,2,3}</td>
<td>92.2</td>
<td>49.2</td>
<td>12.0</td>
<td>20.5</td>
<td>5.5</td>
<td>23.0</td>
<td>18.1</td>
<td>29.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>CP^{2}</td>
<td>91.6</td>
<td>49.3</td>
<td>13.0</td>
<td>18.5</td>
<td>5.9</td>
<td>23.7</td>
<td>19.6</td>
<td>36.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>PP^{2}</td>
<td>83.7</td>
<td>49.5</td>
<td>13.1</td>
<td>13.9</td>
<td>5.2</td>
<td>20.6</td>
<td>17.0</td>
<td>29.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>WP^{1,2,3}</td>
<td>80.2</td>
<td>49.6</td>
<td>12.8</td>
<td>13.3</td>
<td>5.0</td>
<td>20.7</td>
<td>17.7</td>
<td>24.8</td>
<td>0.3</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WG^{1}</td>
<td>102.2</td>
<td>48.7</td>
<td>1.3</td>
<td>18.9</td>
<td>11.4</td>
<td>37.9</td>
<td>30.6</td>
<td>35.7</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>WP^{1}</td>
<td>86.9</td>
<td>49.3</td>
<td>1.4</td>
<td>11.9</td>
<td>10.6</td>
<td>29.0</td>
<td>24.2</td>
<td>26.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Chief</td>
<td>WG^{2}</td>
<td>83.6</td>
<td>48.8</td>
<td>11.8</td>
<td>11.3</td>
<td>4.2</td>
<td>18.1</td>
<td>12.5</td>
<td>19.7</td>
<td>0.3</td>
</tr>
<tr>
<td>CDC Rattan</td>
<td>WG^{2}</td>
<td>95.3</td>
<td>49.0</td>
<td>2.9</td>
<td>18.5</td>
<td>7.5</td>
<td>38.5</td>
<td>29.9</td>
<td>30.5</td>
<td>0.1</td>
</tr>
<tr>
<td>AC Klinck</td>
<td>WG^{2}</td>
<td>85.8</td>
<td>49.1</td>
<td>12.4</td>
<td>11.7</td>
<td>4.8</td>
<td>27.0</td>
<td>21.4</td>
<td>26.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Kawartha</td>
<td>WG^{2}</td>
<td>85.1</td>
<td>49.4</td>
<td>12.4</td>
<td>12.8</td>
<td>4.7</td>
<td>22.8</td>
<td>16.7</td>
<td>28.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Alberta</td>
<td>WG^{2}</td>
<td>91.2</td>
<td>49.2</td>
<td>11.0</td>
<td>13.3</td>
<td>4.4</td>
<td>20.2</td>
<td>13.7</td>
<td>20.9</td>
<td>0.2</td>
</tr>
<tr>
<td>GB</td>
<td>WG^{2}</td>
<td>89.5</td>
<td>49.1</td>
<td>11.6</td>
<td>20.5</td>
<td>6.4</td>
<td>22.2</td>
<td>15.5</td>
<td>19.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*WG = whole grain, CP = commercial pearled, PP = pot pearled and WP = white pearled.

^{1,2,3} Experiments in which the fractions were tested, \( n = 3 \).
Supplemental Figure 1. Blood glucose concentration in healthy participants after the ingestion of white bread and pearled barley cultivars (Expt. 1). Values are means, n=10 (Pooled SE= 0.11)
Supplemental Figure 2. Blood glucose concentration in healthy participants after the ingestion of white bread and different Barley pastas (Expt. 3). Values are means, n=10 (Pooled SE=0.19).
5. Comparison of the effects of Chemical Composition, Processing and Food Form on the Satiety of Barley
5.1. Abstract

Low-glycemic index (GI) foods have been promoted for their ability to potentiate satiety. Barley is suggested to induce satiety; recently a number of barley cultivars have been developed for consumer uses and it has been shown that differences in chemical composition, processing and food-form affects the GI, but the effects on satiety are not known. To investigate these factors on satiety eight cultivars varying in the nature of starch and β-glucan were studied in two experiments in separate groups of 10 subjects. Satiety ratings were collected every 15 min over two hours, satiety area under curve (AUC) and satiety index (SI) calculated. Seven cultivars were tested with one undergoing four levels of pearling. There was a significant main effect on AUC satiety, only two cultivars resulted in a significantly higher AUC satiety than white bread \((F_{9,11} = 2.14, p = 0.04)\) with no differences compared to other cultivars. The rest of the cultivars did not differ significantly than white bread. Pearling did not have an effect on satiety \((p > 0.05)\). Only two cultivars were significantly different than the SI of white bread \((F_{9,10} = 2.25, p = 0.021)\), but not the other cultivars. WG and WP of two cultivars varying in total fibre were made into wet pasta. Compared to white bread, only the high fibre barley pasta had a higher satiety AUC \((F_{9,4} = 4.09, p = 0.004)\). In conclusion, barley cultivars did not affect satiety differently; further studies are required to determine the effect of barley on satiety.
5.2. Introduction

Low-GI diets have been promoted for weight loss and maintenance due to their positive effects on glycemia and on appetite regulation (18, 164, 165). This is consistent in part with the glucostatic hypothesis postulated by Mayer almost 60 years ago (131). The low-GI and satiety hypothesis stipulates that low-GI foods may potentially reduce gastric emptying and lead to a slower absorption producing less variation in glycemic responses which in turn could increase satiety and appetite regulation. However, data from another trial indicate that satiety is no longer influenced by glycemic responses per se 2 hours after the termination of a meal (132). In addition there is further evidence showing early satiating effect of high-GI CHO such as glucose in suppressing short term food intake 60-90 min after the ingestion of a preload compared to a low GI food (133). Moreover, two recent experimental studies found that low-GI test meals had no effect on satiety or food intake (134, 135). On the other hand, low-GI foods are generally high in fiber, which may play a role in appetite regulation (166). In epidemiological studies fiber has been found to be associated with reduced weight gain over time and improved glycemia (136) and in short-term experimental studies fiber has been found to increase satiety and reduce food intake (137-139). But not all studies found an effect; Mattes tested a combination of fiber systems and found no effect of guar and alginate fibers in a solid food matrix on appetite (140). Literature in this area is still inconclusive and warrants further research. Nevertheless, there is interest in finding foods that are slowly digested and increase satiety as alternatives to common refined carbohydrate (CHO) staples such as bread and rice. Such foods have been implicated with the obesity epidemic (167). Barley, a low-GI whole grain holds great potential as an alternative to current grains and as a functional food product due to its high β-glucan fiber content and low GI. Many new barley cultivar varieties have been specifically produced for human consumption with
unique functional characteristics in response to the favorable effects of β-glucan on health and
the recent FDA approval of claims using β-glucan\(^{(24)}\). In a previous study we have shown that
compositional differences in barley cultivars and other factors such as the level of pearling,
amylose-to-amyllopectin ratio, and food form influence post prandial responses of barley and
alter their GI \(^{(168)}\). Therefore, the objective of this study was to investigate the effects of low GI
foods on satiety, specifically to examine the effects of compositional differences, pearling and
food form on the satiety of 9 barley cultivars in healthy participants. This project was completed
in 2 separate experiments. The purpose of study 1 was to examine the effects of composition and
level of pearling (e.g. wholegrain (WG), commercial pearled (CP), pot pearled (PP) and white
pearled (WP)). In study 2 a novel in-house wet pasta-like product was manufactured from 100%
barley flour to assess the effect of food form on satiety.

5.3. Materials and Methods

All experiments were performed using protocols approved by the Research Ethics Board
at the University of Toronto. All participants provided written informed consent to participate.
Two experiments, each with a randomized design were performed in separate groups of 10
healthy participants in the morning after a 10-12 h overnight fast.

Barley Samples

Nine Canadian barley cultivars were selected based on diversity in starch type and β-
-glucan content and for economical purposes and market availability. The selected barley
cultivars included 3 two-row, hulled, normal barleys (AC Parkhill, Chief, GB 992027); 3 six-
rowed, hulled, normal barleys (AC Klink, Celebrity, OAC Kawathra); 1 two-row hull-less,
normal barley (AC Alberta); and 2 two-row, hull-less, waxy barley (CDC Fibar, CDC Rattan).
Kernels from two-row barleys are generally larger and more uniform in size than those from six-rowed barleys due to crowding of spikelet’s on the spike in the latter. Hull-less barley are free-threshing or naked grains. Normal barley contains starch with an amylose-to-amylopectin ratio of $\approx 1:3$, whereas the starch in waxy barleys is almost entirely amylopectin. The amylose-to-amylopectin ratio influences the rate of starch digestion and in turn dictates the concentration of RDS and SDS $^{(156)}$. All cultivars were from Canadian suppliers, samples were provided in 25 kg amounts and a 3 kg representative sample of each cultivar was obtained from the original bags for this study. Barley kernels were pearled in 100g batches for various times to achieve the desired level using an abrasive mill (model TM05, Satake, Tokyo, Japan). Barley fractions used in the current study are presented in Table 1; the entire fractions were described in a previous study $^{(82)}$.

**Experiment 1**

Subjects in Experiment 1 were 6 women and 4 men aged $46.6 \pm 4.4$ y with BMI $26.4 \pm 1.1$ kg/m$^2$. Seven barley cultivars were included (Tables 1); 6 were studied only in the WG state, while 1 cultivar (Celebrity) was pearled to reproduce four fractions: WG, commercial pearled (CP) (some bran and germ were also removed), pot pearled (PP) (all bran and most of the germ removed) and WP. Participants were, therefore, studied on 13 occasions; they consumed 10 different barley test meals in randomized order and white bread 3 times using the same procedures as described for experiment 1 in chapter 4. All subjects were nonrestrictive eaters and were told that the study will examine the effects of different barley cultivars on blood glucose only so not to ponder excessively over their appetite ratings.
Experiment 2

8 women and 2 men aged 40.5 ± 4.7y with BMI 28.3 ± 2.0 kg/m² were recruited for this experiment. The objective was to determine the effect on satiety of making pasta from barley flour, using barley cultivars known to have different GI values (AC Parkhill and Celebrity) each pearled to different levels (WG and WP). Barley samples were milled into flour using an analytical mill (A-10, Tekmar, Cincinnati, OH) and made into a wet pasta-like product using 100% barley flour from each cultivar. Barley flour (100 g) was mixed with water (60-70 mL) to form a paste which was extruded using a pasta maker (PastaMatic® MX700, Treviso, Italy). As a control, wet durum semolina pasta (100 g) mixed with water (35 mL) was manufactured under the same conditions using commercial durum semolina (Robin Hood®, Cargill, Incorporated, Minneapolis, MN). All products were analyzed for dry matter (DM) and wet and dry loss. Salt (1g), xanthan (1g) and 85µL of annato solution (2.8%) were added per 100g barley flour to improve flavor, texture and color, respectively, and overall appearance. After extruding the pasta, products were refrigerated overnight at 4 C° before being cooked on the next morning.

Cooking Procedures

Based on the in vitro carbohydrate analysis, samples of each barley cultivar were cooked in portions that provide 50 g available carbohydrate (Table 1). Single portions of barley grains were rinsed with cold water, added to boiling salted water (1:5 ratio barley: water, w/v), covered and boiled for 25 min (WG and CP fractions) or 30 min (PP and WP fractions), and allowed to sit at room temperature for 10 min prior to being served to the participants. Pasta was cooked in boiling water for 5 min for the barley products and 20 min for the semolina product to obtain
aldente (firm to the bite) texture. A ratio of 1:8 pasta: water, w/v, was used to minimize losses of nutrients due to cooking in large amount of water.

**Satiety Ratings**

Subjective satiety was assessed immediately before each test meal and for 120 minutes after using the tool described by Holt et al\(^{(182)}\) using an equilateral 7-point rating scale which consisted of a 125 mm horizontal line anchored by 7 vertical ticks spaced equally from one end to the other. Above the 7 vertical ticks were printed the following phrases from left to right: ‘extremely hungry’, ‘hungry’, semi hungry’, ‘no particular feeling’, semi satisfied’, ‘satisfied’ and ‘extremely full’. Subjects were asked to make a mark along the horizontal line indicating their satiety at that moment. The 7 rating scales for each experiment were printed on one sheet of paper; thus, while subjects were asked not to discuss their ratings with others, they were able to see their own previous ratings for the current test. Subjective satiety was scored in mm from the left end (extremely hungry) of the scale. Immediately after they finished eating, the subjects recorded the time taken to eat the test meal and then they rated: how much they liked the test meal. A satiety index score (SI) was calculated as described by Holt et al\(^{(185)}\). Palatability was rated on a 100 mm scale ranging from ‘very unpalatable’ to ‘very palatable’

**Statistical Analysis**

Results are expressed as means ± SEM. Statistical analysis was done using SAS version 9.3. Incremental areas under the satiety response curve (iAUC), ignoring area below the baseline, were calculated using the trapezoid rule as previously described\(^{(111)}\). Satiety index scores (SI) were obtained by dividing the satiety AUC value for the test food by the mean of
each subject’s response to WB and expressed as a percentage according to the methods described in Holt et al. The SI equation is as follows:

\[
SI \text{ Score } (\%) = \frac{120 \text{ min } Satiety \text{ AUC for the test Barley product}}{120 \text{ min } Satiety \text{ AUC for the Reference food}} \times 100 \%
\]

Fasting satiety AUC values were adjusted using regression analysis. To determine the significance of the differences in Satiety AUC values for all test meals a repeated measures analysis of variance (ANOVA) was employed. Similar analysis was repeated for the SI and palatability values. To determine the effects of cultivar and pearling in experiments 1 and 2 the mean satiety iAUC values of the barley test meals (not including white bread controls) were subjected to repeated measures ANOVA to determine the main effects of cultivar and level of pearling and the cultivar × pearling interaction. In all cases, after demonstration of significant heterogeneity by ANOVA, differences between individual means was assessed using Tukey’s test to adjust for multiple comparisons.

5.4 Results

The average changes in satiety AUC over 120 minutes following the consumption of different test barleys are illustrated in figure 1 and 2. The AUC satiety and SI scores are shown in table 2 and 3. There was a significant main effect on AUC satiety, CDC-Rattan and Kawartha were significantly more satiating than white bread \((F_{9,11} =2.14, p =0.04)\) with no differences compared to other cultivars. There were no significant differences between the other cultivars and white bread. Post-hoc analysis of the effect of level of pearling on Celebrity cultivar and its fractions showed no significant differences (AUC Celebrity WG = 6105 ± 1074 mm vs. AUC Celebrity WP = 6078 ± 1074 mm, \(p > 0.05\)). All the barley cultivars had a higher
SI compared to WB (range 133-175%) and only CDC-Rattan and Kawartha were significantly different than the SI of white bread ($F_{9,10} = 2.25, p = 0.021$).

In experiment 2 similar results were seen, there was a main effect of differences between the treatments, compared to white bread both Celebrity and Semolina pasta had a higher AUC (AUC WB = 5011 ± 875 mm vs. AUC Celebrity WG = 7118 ± 839 mm, $F_{9,4} = 4.09, p = 0.004$); AC-Park Hill pasta was not significantly differences than white bread or the other pastas (Table 2). The Satiety index of Celebrity and Semolina pasta was also significantly different than that of white bread but not AC-Park Hill pasta ($F_{9,5} = 3.26, p = 0.01$).

In experiment 1 palatability ratings did not differ between cultivars or compared to white bread. In experiment two, the WP celebrity pasta was significantly less palatable than its respective WG fraction ($p = 0.002$). There were no differences between the other pastas compared to semolina pasta or white bread. Palatability was not associated with satiety AUC in both the cooked barley kernels ($p = 0.99$) and Barley Pasta ($p = 0.75$). There were no significant correlations between satiety AUC, SI and either AUC of blood glucose (data not shown) or the GI.

5.5. Discussion

These results offer estimates of visual and orosensory perceptions. These cues provide important information to factors that contribute to appetite regulation. They suggest, at least as measured by the instrument used, that cooked whole grain barley kernels and its pearled fractions do not potentiate satiety differently than a high-GI food (white bread). Our data are in agreement with previous reports that satiety and feelings of fullness are not part of the mechanism by which low-GI foods regulate appetite or weight maintenance ($^{134, 135}$). In
experiment one only two cultivars (CDC-Rattan and Kawartha) were more satiating than the reference food, white bread, but not compared to the other cultivars. This could be due to the fact that the cultivars did not differ in their GI values and may potentiate satiety equally but in this case it may be other factors that are involved in potentiating satiety such as food appearance and food volume. Nonetheless, studies examining the effect of boiled barley kernels per se on satiety are scarce and limited, where most studies have examined barley as a food component rather than whole intact grain. Moreover, Barley studies are further complicated by differences in chemical composition and food preparation methods. For example, Granfeldt et al (102) fed four different barley genotypes as whole kernels and milled flour (Porridge) and compared their effect on satiety to white bread. All barley products produced a significantly higher satiety compared to white bread. The results from that study are not consistent with ours; however, the discrepancy could be explained by the differences in resistance starch (RS) content. In our study RS was negligible ranging from 0.11 - 0.49 g/serving compared to 2.0 -3.0 g/serving in the Granfeldt et al study. Similar results and explanation apply to Liljeberg et al (141) and Nilsson et al (142). Kaplan and Greenwood (143) investigated the effects of barley on satiety compared to a placebo drink, glucose drink and instant mashed potatoes in an elderly population. Compared to the placebo and the glucose drinks barley and mashed potatoes elicited a higher satiety scores (P <0.008), with the mashed potatoes increasing satiety significantly more than barley (P = 0.015) despite it being a high GI food. Moreover, Barley in this study was consumed with 2.5 g of butter which may have led to higher satiety compared to placebo and glucose drinks due to a slower gastric emptying by virtue of fat. The reference food in that study may also be an issue as liquids are known to be less satiating than solid foods (169, 170). Finally in Kaplan and Greenwood study they observed that by 120 min post ingestion, barley no longer produced higher satiety
than glucose and placebo. However, our results are in line with studies examining barley incorporated in different food products (171, 172).

In experiment two results were slightly different as there was a main effect on satiety after the consumption of barley pasta made from the high-fibre cultivar (Celebrity). Pasta made from the Celebrity cultivar was more satiating compared to white bread (P = 0.004). These results are not surprising; food form and volume may have contributed to these effects. Barley pasta had a larger volume and weight per serving (224 g) compared to the same cultivar in Kernel form (92 g). These results are in line with studies that fed mixed meals with differing GI indices to examine their effect on satiety. For example, Liu et al (135) fed mixed meals differing in GI and CHO content and found no differences in ratings of hunger, fullness or satiety between the meals. Similar results and conclusion were also shown by Aston et al (134) and Gelibter et al (173). The notion that low-GI foods confer effects on satiety or appetite regulation stem partly from the glucostatic theory. The theory postulates that appetite increases when the blood glucose concentration falls. Wolever et al (132) examined the effects of day to day variations in glycemic responses after feeding different portions of white bread, there were significant differences in satiety but these differences were not related to blood glucose concentrations. The barleys used in this study had very low RS content which may be responsible for potentiating satiety in previous studies. Nonetheless, all cultivars had very low GI values and were shown to elicit lower glycemic responses compared to WB in previous studies (168). Objective measures of satiety such as gut hormones and measures of energy intake would have provided valuable data to this project. This study did not have statistical power or an adequate number of subjects to measure satiety, nonetheless, it provide a valuable insight to the satiety index and its relationship to the low-GI and satiety hypothesis. In conclusion, boiled barley kernels with similar GI values
do not have a significant effect on satiety that is beyond the effect of white bread - a high-GI food. Barley pasta with higher total fibre content potentiate satiety more than the low fibre one regardless of pearling indicating a potential interaction between food form and fibre content. Our results do not support the hypothesis that low-GI foods increase satiety or have an effect on appetite. Satiety index appears to have no associated with glycemic responses. Further studies with are required to confirm the effect of barley on satiety.
Table 1. Average values for Starch characteristics of barley eight cultivars and their fractions (g/serving)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fraction*</th>
<th>Serving</th>
<th>Total Starch (g)</th>
<th>Amylose (g)</th>
<th>Total Fiber (g)</th>
<th>β-glucan (g)</th>
<th>RAG (g)</th>
<th>RDS (g)</th>
<th>SDS (g)</th>
<th>RS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-Parkhill</td>
<td>WG²</td>
<td>87.6</td>
<td>49.3</td>
<td>11.8</td>
<td>16.0</td>
<td>3.8</td>
<td>18.6</td>
<td>14.4</td>
<td>19.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>WP²</td>
<td>76.1</td>
<td>49.7</td>
<td>12.4</td>
<td>10.0</td>
<td>3.4</td>
<td>17.5</td>
<td>15.0</td>
<td>19.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Celebrity</td>
<td>WG¹²</td>
<td>92.2</td>
<td>49.2</td>
<td>12.0</td>
<td>20.5</td>
<td>5.5</td>
<td>23.0</td>
<td>18.1</td>
<td>29.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>CP¹</td>
<td>91.6</td>
<td>49.3</td>
<td>13.0</td>
<td>18.5</td>
<td>5.9</td>
<td>23.7</td>
<td>19.6</td>
<td>36.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>PP¹</td>
<td>83.7</td>
<td>49.5</td>
<td>13.1</td>
<td>13.9</td>
<td>5.2</td>
<td>20.6</td>
<td>17.0</td>
<td>29.4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>WP¹²</td>
<td>80.2</td>
<td>49.6</td>
<td>12.8</td>
<td>13.3</td>
<td>5.0</td>
<td>20.7</td>
<td>17.7</td>
<td>24.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Chief</td>
<td>WG¹</td>
<td>83.6</td>
<td>48.8</td>
<td>11.8</td>
<td>11.3</td>
<td>4.2</td>
<td>18.1</td>
<td>12.5</td>
<td>19.7</td>
<td>0.3</td>
</tr>
<tr>
<td>CDC Rattan</td>
<td>WG¹</td>
<td>95.3</td>
<td>49.0</td>
<td>2.9</td>
<td>18.5</td>
<td>7.5</td>
<td>38.5</td>
<td>29.9</td>
<td>30.5</td>
<td>0.1</td>
</tr>
<tr>
<td>AC Klinck</td>
<td>WG¹</td>
<td>85.8</td>
<td>49.1</td>
<td>12.4</td>
<td>11.7</td>
<td>4.8</td>
<td>27.0</td>
<td>21.4</td>
<td>26.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Kawartha</td>
<td>WG¹</td>
<td>85.1</td>
<td>49.4</td>
<td>12.4</td>
<td>12.8</td>
<td>4.7</td>
<td>22.8</td>
<td>16.7</td>
<td>28.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Alberta</td>
<td>WG¹</td>
<td>91.2</td>
<td>49.2</td>
<td>11.0</td>
<td>13.3</td>
<td>4.4</td>
<td>20.2</td>
<td>13.7</td>
<td>20.9</td>
<td>0.2</td>
</tr>
<tr>
<td>GB</td>
<td>WG¹</td>
<td>89.5</td>
<td>49.1</td>
<td>11.6</td>
<td>20.5</td>
<td>6.4</td>
<td>22.2</td>
<td>15.5</td>
<td>19.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

¹,² Experiments in which the fractions were tested, n = 3. RAG: Rapidly available glucose. RDS: Rapidly digested starch. SDS: Slowly digested starch, RS: Resistant Starch
Table 2. Incremental areas under the satiety curve (iAUC) and satiety Index (SI) of cooked barley cultivars and white bread, Expt. 1

<table>
<thead>
<tr>
<th>Barley Cultivar</th>
<th>Satiety iAUC mm</th>
<th>Satiety Index score (SI)</th>
<th>iAUC Blood Glucose (mmol×min/L)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celebrity WG*</td>
<td>6105 ± 1074 ac</td>
<td>133 ± 19.8 ac</td>
<td>48±9 a</td>
<td>21 ± 4 a</td>
</tr>
<tr>
<td>Celebrity CP*</td>
<td>6234 ± 640 ac</td>
<td>153 ± 37.5 ac</td>
<td>55±6 a</td>
<td>25 ± 3 a</td>
</tr>
<tr>
<td>Celebrity PP*</td>
<td>6342 ± 865 ac</td>
<td>135 ± 18.3 ac</td>
<td>47±5 a</td>
<td>22 ± 3 a</td>
</tr>
<tr>
<td>Celebrity WP*</td>
<td>6078 ± 1074 ac</td>
<td>134 ± 23.5 ac</td>
<td>68±11 a</td>
<td>32 ± 6 a</td>
</tr>
<tr>
<td>Alberta</td>
<td>5768 ± 805 ac</td>
<td>120 ± 19.8 ac</td>
<td>63±13 a</td>
<td>29 ± 4 a</td>
</tr>
<tr>
<td>Chief</td>
<td>7004 ± 1054 ac</td>
<td>165 ± 35.3 ac</td>
<td>65±11 a</td>
<td>26 ± 6 a</td>
</tr>
<tr>
<td>CDC Rattan</td>
<td>6812 ± 1161 ab</td>
<td>133 ± 19.3 ab</td>
<td>58±12 a</td>
<td>36 ± 8 a</td>
</tr>
<tr>
<td>AC Klinck</td>
<td>6075 ± 1183 ac</td>
<td>133 ± 24.3 ac</td>
<td>71±12 a</td>
<td>28 ± 4 a</td>
</tr>
<tr>
<td>Kawartha</td>
<td>7518 ± 563 ab</td>
<td>175 ± 27.9 ab</td>
<td>61±10 a</td>
<td>29 ± 7 a</td>
</tr>
<tr>
<td>GB</td>
<td>6445 ± 653 ac</td>
<td>137 ± 6.7 ac</td>
<td>52±10 a</td>
<td>24 ± 5 a</td>
</tr>
<tr>
<td>White bread</td>
<td>4774 ± 478 c</td>
<td>100 c</td>
<td>163±15 b</td>
<td>71 b</td>
</tr>
</tbody>
</table>

* Values are means ± SEM for n =10. Means with different superscript letters differ significantly (Repeated measures of analysis, post hoc Tukey test, P < 0.05.) * Celebrity Cultivar has been pearled into 4 levels of pearling; WG: Whole grain, CP: Commercial pearl, PP: Pot pearled, WP: White pearled.
Table 3. Incremental areas under the satiety curve (iAUC), Satiety index (SI), iAUC of the glycemic response and the GI of two barley pastas and their fractions, semolina pasta and white bread, Expt. 2.\textsuperscript{§}

<table>
<thead>
<tr>
<th></th>
<th>Satiety iAUC mm</th>
<th>Satiety Index Score (SI)</th>
<th>iAUC Blood Glucose (mmol×min/L)</th>
<th>GI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fraction\textsuperscript{e}</td>
<td>Mean</td>
<td>Fraction</td>
<td>Mean</td>
</tr>
<tr>
<td>Celebrity</td>
<td>WG 7118±835 WP 7365±945</td>
<td>7241±890\textsuperscript{abc}</td>
<td>140±20.9</td>
<td>151±27.0</td>
</tr>
<tr>
<td>AC-Parkhill</td>
<td>WG 6574±1166 WP 6239±898</td>
<td>6406±103\textsuperscript{ab}</td>
<td>121±13.4</td>
<td>118±12.7</td>
</tr>
<tr>
<td>Mean</td>
<td>WG 6846±1000 WP 6802±921</td>
<td>130±17.5</td>
<td>134±19.8</td>
<td>139±14</td>
</tr>
<tr>
<td>Semolina</td>
<td></td>
<td>8379±706\textsuperscript{c}</td>
<td>178±26.7\textsuperscript{a}</td>
<td>151±20</td>
</tr>
<tr>
<td>White Bread</td>
<td></td>
<td>5011±875\textsuperscript{b}</td>
<td>100\textsuperscript{a}</td>
<td>142±16</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Values are means ± SEM for n = 10.
\textsuperscript{abc}Means with different superscript letters differ significantly (Repeated measures of analysis, post hoc Tukey test, P < 0.05.)
\textsuperscript{e}WG: wholegrain; WP: white pearled.
\textsuperscript{§}Pasta made from 2 barley cultivars pearled in 2 different ways and semolina pasta as a control
\textsuperscript{a}Significant main effect of pearling level, P < 0.05.
Figure 1. Average satiety AUC for healthy subjects after the consumption of 10 different barley cultivars and the reference food (white bread). Values are means, n=10. Expt 1.
Figure 2. Average satiety AUC for healthy subjects after the consumption of 4 different barley Pastas and the reference food (white bread). Values are means, n=10. Expt 2
6. The Relationship between the Physico-Chemical Characteristics of Barley and Postprandial Glycemia and the Glycemic Index
6.1. Abstract

Recently a number of governmental regulating agencies have approved food health claims for barley. This has prompted the development of barley cultivars specifically for human consumption. Differences in chemical composition, processing method and food-form affect post-prandial glycemia and glycemic index (GI), but it is unknown whether this is related to differences in physico-chemical properties. To investigate this, 3 cultivars varying in nature of starch and β-glucan content, which have been tested previously in a clinical trial to determine their GI, were analyzed to characterize their molecular weight (MW), solubility and β-glucan viscosity in an in vitro digestive model. 12 cooked barley products were tested either in boiled grain form (rice replacement) or a fresh pasta-like product and each cultivar was pearled into either a whole grain or white pearled fraction. β-glucan content did not differ significantly between kernels and pastas but MW, viscosity, β-glucan contribution to viscosity, and solubility were significantly reduced in the fresh barley pasta products compared to the grains. Peak blood glucose rise (PBGR) and the GI were correlated with log MW (PBGR, \( r = -0.83, P = 0.003 \), GI, \( r = -0.72, P = 0.018 \)) and log β-glucan viscosity (PBGR, \( r = -0.85, P = 0.002 \), GI, \( r = -0.80, P = 0.005 \)). The GI of barley is inversely related to β-glucan viscosity which depends on its MW. Pearling does not affect the physico-chemical characteristics significantly, but milling and extruding barley grains to create fresh barley pasta resulted in a sharp reduction in MW and viscosity.
6.2. Introduction

Barley has received abundant interest in the past decade not only as a functional food ingredient but also as a whole grain substitute to current highly refined grains. This interest is due in part to barley’s high content of the soluble fibre β-glucan. Several studies have shown that the mixed linkage (1→3, 1→4)-β-D-glucan has the capacity to attenuate postprandial responses of glucose, insulin and blood lipids in human subjects (28, 174). Most recent research suggests that it may also have favorable effects on satiety inducing gut hormones (175). The physiological effects of β-glucan on a number of postprandial markers have been mostly attributed to its ability to increase viscosity in the digesta thereby modifying starch digestibility and retarding glucose absorption (28). In view of that, various food and health organizations have approved health claims for barley, the first of which was in the United States (24). More recently, Health Canada (25) and the European Food Safety Authority (EFSA) (176) have approved similar health claims for barley. All of these health claims associate the consumption of barley β-glucan with a reduction of cholesterol except for the EFSA claim which includes the maintenance of normal glycemia.

The magnitude of these physiological effects depends on β-glucan viscosity which in turn depends on the physico-chemical properties of the β-glucan in food. This has been demonstrated for β-glucan capacity to reduce serum cholesterol and low-density lipoprotein (LDL). Wolever et al (72) conducted a double blind parallel design randomized trial to determine the physiological effectiveness of high-MW vs. medium and low-MW oat β-glucan cereal on lowering LDL cholesterol in 345 hypercholesterolemic subjects. The results show that when subjects with high LDL cholesterol consumed a breakfast meal containing a 3 g high or medium-MW oat β-glucan cereal, LDL was lowered by 5% but the effect was reduced by 50% in subjects consuming a breakfast meal with a low-MW oat β-glucan. This relationship has been established for foods
enriched with β-glucan, mainly from oat, such as muffins and ready-to-eat (RTE) cereals (75, 76, 158, 177) but surprisingly not for barley or for β-glucan in its naturally occurring state within the intact kernel.

The consumption of barley grains as a rice substitute requires processing for a number of reasons including: meeting consumer demand for a white bright grain colour, increasing the palatability and/or producing fractions with high β-glucan. Yet, the effects of different levels of pearling, a common commercial process whereby the husk and outer layers of barley grains are removed by a friction and abrasion process, on barley physico-chemical properties have not been comprehensively examined; in particular the association between the physico-chemical products and postprandial responses. In RTE oat based cereals different methods of processing can be employed to manipulate the physico-chemical properties including MW and viscosity. Tosh et al (76) modified MW and solubility by extrusion cooking of oat bran RTE cereals. The authors noted that reducing MW coincided with an apparent drop in viscosity in an in vitro digestion model. We have previously shown that differences in chemical composition between cultivars, pearling and changing the food form of barley kernels results in significantly different post prandial blood glucose responses and alter the glycemic index (GI) significantly (168). Whether these effects are due to changes in the physico-chemical properties are not known.

Barley β-glucan has not been extensively studied in comparison to oats and recently a number of barley cultivars have been produced for human consumption. Data are limited when it comes to characterising the physico-chemical properties of different barley cultivars. The effects of processes like pearling and milling on the physico-chemical properties is similarly underappreciated, whether the differences in physico-chemical properties will have a physiological effect on glycemia and the GI is not yet known. The objectives of this study were:
1) characterize the physico-chemical properties of 3 different barley cultivars; 2) assess the impact of pearling on the physico-chemical properties of each cultivar; 3) examine the effects of milling and extrusion cooking on the physico-chemical properties and their corresponding physiological responses, and finally, (4) assess the relationship between the physico-chemical properties and postprandial glycemia and the GI. The main outcomes include: MW, viscosity, solubility, β-glucan content, blood glucose and the GI.

6.3. Materials and Methods

Experimental foods

Three Canadian barley cultivars were selected based on diversity in starch, amylose, β-glucan content and for agronomic purposes. The cultivars chosen were AC Parkhill (high amylose, low β-glucan), Celebrity (high amylose, medium β-glucan) and CDC Fibar (low amylose, high β-glucan) (Table 1). Barley kernels were pearled in 100g batches at various times to achieve the desired pearling level using an abrasive mill (model TM05, Satake, Tokyo, Japan). Each cultivar was pearled into two fractions, wholegrain (WG) (only the husk was removed) and white-pearled (WP) (all the bran and most of the germ and crease removed). Botanically the selected barley cultivars were: 1, two-row, hulled, normal barley (AC Parkhill); 1, six-rowed, hulled, normal barley (Celebrity); and 1 two-row, hull-less, waxy barley (CDC Fibar). Kernels from two-row barleys are generally larger and more uniform in size than those from six-rowed barleys due to crowding of spikelet’s on the spike in the latter. Hull-less barley are free-threshing or naked grains. Normal barley contains starch with an amylose-to-amylopectin ratio of ≈ 1:3, whereas the starch in waxy barleys is almost entirely amylopectin. Samples were provided in 25
kg amounts and a 3 kg representative sample of each cultivar was obtained from the original bags.

**In vitro digestion and viscosity measurement**

Each test food was prepared and cooked according to the same condition of the clinical trials (168). The Rapid Visco Analyzer (RVA) method previously described (178) was used for digestion and direct measurement of β-glucan viscosity. Cooked barley grains and pasta were milled using a M2 universal mill (IKA-Werke, Staufen, Germany), and a portion of sample containing 0.25g β-glucan content (dry base) was weighed into the RVA canister. Sodium phosphate buffer (20 mM, pH 6.9) containing 10 mM NaCl was added to the canister with 100 μL of human saliva amylase EC.3.2.1.1. (Sigma/Aldrich A1031) (300 U/mL in 3.2 mM CaCl₂ solution), 300 μL of pepsin EC. 3.4.23.1 (Sigma/Aldrich P7012) (1130 U/mL in 0.9% NaCl solution) and 600 μL of pancreatin (Sigma/Aldrich P7545) (0.5 mg/mL in sodium phosphate buffer). The amount of buffer was calculated as 100 g minus the weight of sample moisture. The RVA (Model RVA-4, Newport Scientific Pty Ltd, Australia) equipped with thermocline software version 2.2. for windows was held constant at 37°C, and the mixing speed was set at 480 rpm for 10 sec followed by 160 rpm during the run. Viscosity, was recorded every 8 sec during the 2 hour digestion.

**β-Glucan content, molecular weight, solubility and extract viscosity**

Total β-glucan content was determined by the enzymatic method (AOAC 995.16). The amount of extractable β-glucan obtained after digestion and centrifugation at 8000×g and 20°C for 10 minutes was measured using flow-injection analysis (FIA) as described previously (76). The percent solubility was calculated as amount of soluble β-glucan/ total β-glucan × 100. The
weight average molecular weight ($M_w$) was determined using size exclusion-HPLC with calcofluor detection as previously described (76). A controlled strain rheometer (ARES, TA Instruments, New Castle, DE) was used to measure the viscosity of extracts after digestion and centrifugation at 8000×g and 20°C for 10 minutes. Up and down shear ramps were conducted over a shear rate range of 0.1 - 400 s$^{-1}$ at 37 °C using a cone-and-plate geometry (angle of 0.04 radians, diameter of 50 mm). Apparent viscosity was recorded at a shear rate 30 s$^{-1}$ on down cycle, primarily for consistency with the literature.

**Carbohydrate Analysis**

The methods and results of carbohydrate analysis have been reported previously (82) and will only be used in this study for correlation analyses with the in vivo glycemic responses, the in vitro digestion model and the physico-chemical data.

**Clinical Trial**

The results from the clinical trials are given in chapter 4. The blood glucose (BG), area under the curve (AUC) and GI values were used to assess the association with the physico-chemical properties. The trial was conducted in two experiments with 10 separate subjects for each trail. In study 1: Six of the test meals consumed by each subject consisted of the 3 different cultivars of barley as explained previously and were cooked as described below and fed in randomized order. In study 2: The objective was to determine the effect on the GI of making pasta from barley flour, using two barley cultivars shown in experiments 1 to have different GI values (AC Parkhill and Celebrity) each pearled to different levels (WG and WP). Barley samples were milled into flour using an analytical mill (A-10, Tekmar, Cincinnati, OH) and made into a wet pasta-like product using 100% barley flour from each cultivar. Barley flour
(100 g) was mixed with water (60-70 mL) to form a paste which was extruded using a pasta maker (PastaMatic® MX700, Treviso, Italy). All products were analyzed for dry matter (DM) and wet and dry loss. Salt (1g), xanthan (1g) and 85µL of annato solution (2.8%) were added per 100g barley flour to improve flavor, texture and color, respectively, and overall appearance. After extruding the pasta, products were refrigerated overnight at 4 C° before being cooked on the next morning.

Cooking Procedures

Based on the in vitro carbohydrate analysis, samples of each barley cultivar were cooked in portions that provide 50 g available carbohydrate (Table 1). Single portions of barley grains were rinsed with cold water, added to boiling salted water (1:5 ratio barley: water, w/v), covered and boiled for 25 min (WG and CP fractions) or 30 min (PP and WP fractions), and allowed to sit at room temperature for 10 min prior to being served to the participants. Pasta was cooked in boiling water for 5 min for the barley products and 20 min for the semolina product to obtain aldente (firm to the bite) texture. A ratio of 1:8 pasta: water, w/v, was used to minimize losses of nutrients due to cooking in large amount of water.

Statistical Analysis

Results are expressed as mean ± SD. Statistical analysis was done using SAS version 9.3. β-glucan content and the physico-chemical data ( solubility, extract viscosity and MW) of all treatments were compared by a multivariate analysis of variance (MANOVA) examining the effects of cultivar, pearling and food form. After demonstration of significant heterogeneity by ANOVA, differences between individual means was assessed using Tukey's test to adjust for multiple comparisons. Peak Blood Glucose Rise (PBGR) was calculated as the difference of each
subject’s peak and fasting glucose values. Pearson correlation analysis was used to identify relationships between physico-chemical data and BG, AUC and the GI values. The criterion used for statistical significance was 2-tailed $P < 0.05$.

6.4. Results

Physico-chemical data

Boiled barley kernels

β-glucan content of the cooked barley kernels ranged from $4.2 \pm 0.04$ to $11.1 \pm 0.4$ (% db) (Table 2). The waxy cultivar CDC-Fibar had significantly higher β-glucan content compared to the 2-row hulled, normal cultivar AC-Parkhill and the hulled normal cultivar Celebrity ($P < 0.05$), with no cultivar×fraction interactions for all the boiled barley kernels. β-glucan MW ranged from $1460 \pm 320$ to $2310 \pm 600$ (g/mol×$10^3$). The MW did not differ significantly between the boiled barley kernels with no cultivar×fraction interaction. There was a trend for a decline in MW within each cultivar for boiled barleys when pearled but without any statistical differences. Solubility was significantly different between cultivars; CDC-Fibar had higher solubility compared to Celebrity and AC-Parkhill ($P < 0.05$) with no cultivar×fraction interaction. β-glucan Viscosity differed between barley kernels, CDC-Fibar was significantly different than AC-Parkhill but not from Celebrity with no cultivar×fraction interactions ($P < 0.05$). Similarly, extract viscosity differed between cultivars, CDC-Fibar was significantly different then AC-Parkhill ($P < 0.05$) (Table 2). There was a trend for increasing viscosity with pearling, yet not statistically significant. There were also apparent differences in the percent contribution of β-glucan to viscosity between cultivars, though not consistent (Table 2).

Barley Pasta
β-glucan content in the different cooked barley pastas ranged from 4.0 ± 0.01 to 11.30 ± 0.89 (% db) for the barley pasta (Table 3). CDC-Fibar had significantly higher β-glucan content compared to Celebrity and AC-Parkhill cultivars (P < 0.05), with no cultivar×fraction interactions for barley pasta. β-glucan MW ranged from 250 ± 280 to 1240 ± 130 (g/mol×10³) for barley pasta. Comparing the barley pasta physico-chemical properties between cultivars yielded similar observation to the boiled barley kernels. β-glucan MW differed significantly between the barley pastas; CDC-Fibar was significantly different than AC-Parkhill but not Celebrity (P < 0.05). When compared to boiled barley kernels, the MW of barley pasta has significantly declined by approximately 32% to 55% (P < 0.05, table 3). Solubility differed significantly between cultivars, Pasta made from CDC-Fibar cultivar had higher % soluble β-glucan compared to pasta made from Celebrity but not AC-Parkhill (P < 0.05) with no cultivar × fraction interaction. Viscosity differed between barley Pastas, CDC-Fibar was significantly different than AC-Parkhill but not from Celebrity with no cultivar×fraction interactions (P < 0.05). Similarly, extract viscosity differed between cultivars, CDC-Fibar was significantly different then AC-Parkhill (P < 0.05) (Table 3). Similar to the boiled barley kernels the percentage of viscosity contributed by β-glucan in the barley pastas varied and was not consistent.

**Correlation Analysis**

There were significant correlations between log₁₀ [MW] and overall log₁₀ viscosity (r = 0.83, n = 10, P = 0.00), log₁₀ [in vitro extract viscosity] (r = 0.85, n = 10, P = 0.00) and log₁₀ [β-glucan percent contribution of viscosity] (r = 0.76, n = 10, P = 0.01). Additionally, there were negative correlations between log₁₀ [MW] and the GI (r = -0.83, n = 10, P = 0.00) and PBGR (r = -0.72, n = 10, P = 0.02). PBGR was correlated the GI (r = 0.92, n = 10, P = 0.00) and negatively
correlated with overall log$_{10}$ viscosity ($r = -0.76$, $n = 10$, $P = 0.01$), log$_{10}$ [in vitro extract viscosity] ($r = -0.80$, $n = 10$, $P = 0.00$) and log$_{10}$ [$\beta$-glucan percent contribution of viscosity] ($r = -0.74$, $n = 10$, $P = 0.01$). Similarly, the GI was also negatively correlated with overall log$_{10}$ viscosity ($r = -0.79$, $n = 10$, $P = 0.00$), log$_{10}$ [in vitro extract viscosity] ($r = -0.80$, $n = 10$, $P = 0.00$) and log$_{10}$ [$\beta$-glucan percent contribution of viscosity] ($r = -0.78$, $n = 10$, $P = 0.00$).

### 6.5. Discussion

The three boiled barley cultivars: Celebrity, AC-Parkhill and CDC-Fibar had a MW values (Mean ± SD) of $2310 \pm 600 \times 10^3$ (g/mol), $1460 \pm 320 \times 10^3$ (g/mol) and $2290 \pm 105 \times 10^3$ (g/mol) for the WG fractions and $1870 \pm 200 \times 10^3$ (g/mol), $1640 \pm 655 \times 10^3$ (g/mol) and $1960 \pm 140 \times 10^3$ (g/mol) for the WP fractions of each cultivar. MW values were slightly higher than values found in the literature ($^{77}$). The cultivars did not differ significantly in their MW and pearling had no effect on any of the physico-chemical characteristics (Table 2). In the case of AC-Parkhill, pearling tended to increase the MW, the WP fraction of that cultivar had higher mean MW compared to the WG but the difference was not statistically significant. $\beta$-glucan content differed significantly between the cultivars, CDC-Fibar has significantly more $\beta$-glucan than both the other cultivars and pearling had no effect on $\beta$-glucan content. These results suggest that pearling is a mild process that causes little impact on the physico-chemical properties. However, in a previous study we have shown that pearling can increase both the AUC and the GI by 20% and 10% respectively ($^{168}$) and these results suggest that the increase in GI is not due to changes in the physico-chemical properties of the $\beta$-glucan but maybe due to the interaction between the inherent differences in chemical composition and pearling.
Similar results were observed for solubility, the cultivars differed significantly with no pearling effects. CDC-Fibar had significantly higher solubility compared to the other two cultivars which may be due to the significantly higher content of β-glucan. This observation is to be expected as solubility is a function of MW\(^{158}\), and since MW was not affected significantly by pearling therefore, solubility was not affected.

Viscosity of β-glucan, a measure of the resistance to flow\(^{21}\), is an important attribute of determining the quality of β-glucan and its considered to play a significant role in the health benefits associated with consuming barley and barley β-glucan products. Our results show that cultivars differed significantly in terms of the viscosity their constituents induced (Table 2). These results are partly in line with previous work. Gray et al\(^{179}\), found that food product viscosity of the same barley cultivars was significantly affected by both cultivar and pearling level. In this study there was a trend of increasing viscosity with the WP fractions although not significant but this indicates that pearling may result in more accessible and bioactive β-glucan leading to increased viscosity. β-glucan is mainly a soluble fibre but typically only a portion of the total is extracted under physiological conditions. Pearling reduced the viscosity of the β-glucan extract in only Celebrity regardless of retaining the overall viscosity (Table 2). This indicates that other components may be responsible for the developed viscosity. The opposite occurred in CDC-Fibar and AC-Parkhill where pearling increased the viscosity of the extract, differences were not significant (Table 2). These results suggest that cultivars have different rheological behaviors. This may be due to differences in their botanical anatomy (2-row vs. 6-row) and nature of starch (Normal vs. Waxy). For example, Celebrity a 6-row normal hulled cultivar had a significantly more total starch in the WP fraction compared to the WG fraction\(^{82}\) which explains the decline in β-glucan contribution to viscosity in the WP fraction. During
heating at a constant shear rate and temperature, the starch granules swell, rupture and release their content which parallels an increase in viscosity of the starch suspension \(^{(179)}\). In contrast, CDC-Fibar is a waxy cultivar with low starch and high β-glucan content which makes it more resistant to processing effects on β-glucan thereby retaining β-glucan contribution and ability to create a viscous solution in the digesta. Indeed, CDC-Fibar maintained the same percent contribution to viscosity by β-glucan in both fractions (45%, Table 2).

Despite the growing interest in barley as a rice substitute and as a food ingredient – there has been insufficient investigation concerning the effects of processes like pearling, milling and extruding on the physico-chemical properties of cooked barley products. Health Canada has recently approved a food health claim for barley and barley β-glucan as well as the European Food Safety Authority (EFSA) combined with the increased interest in whole grains, consumer demand for barley based products will no doubt increase. Pearled barley is commercially available and our results show that regardless of pearling level, barley grains still retain most of their physico-chemical properties, and in some cultivars it may increase their bioactivity.

On the other hand, when the same barley cultivars and fractions were milled into flour and extruded to create fresh pasta like product made from 100% barley the physico-chemical properties deteriorated significantly despite having the same β-glucan content (Table 3). The MW of the cooked barley pastas decreased by 45 – 56% compared to the cooked barley grains. Solubility was not affected greatly with the exception of the waxy cultivar CDC-Fibar (Table 3). Viscosity has declined sharply in the pasta products compared to the grains by approximately 50 - 90%. In our previous work we showed that these fresh barley pasta products had a relatively medium to high GI compared to conventional dry pasta. The massive decrease in the physico-chemical properties explains these high-GI values. High GI diets have been associated with
increased risk of developing a number of chronic diseases such as type 2 diabetes, cardiovascular disease and cancer \(^{(10)}\). They have also been implicated in reducing satiety and increasing inflammatory responses which may increase the risk of weight gain and developing obesity \(^{(165)}\). In fact, a high GI meal acutely increased postprandial inflammatory responses compared to a low-GI meal in lean healthy young subjects \(^{(180)}\).

We found significant correlations between PBGR, GI and the physico-chemical properties. PBGR, defined as the difference of each subject’s peak and fasting glucose rise, appears to be a more relevant indicator of the physiological impact of β-glucan on glycemia. This reflects the importance of considering the physico-chemical properties of barley and barley β-glucan in food product development. In our previous study we showed that the GI of pasta made from low-GI barley grains increased by 184% in comparison to the GI of its native grain (GI: 25 vs. 71). Both PBGR and the GI were negatively correlated with MW, viscosity and the extract viscosity, results consistent with relationships established in other studies \(^{(158, 177)}\). Similarly, both the GI and PBGR were correlated with the percent contribution of β-glucan to viscosity. This suggests that not only β-glucan content should be considered but its physiological effectiveness to the development of a viscous solution in the gut. Solubility was not correlated with either the GI or PBGR, a result that has been observed in previous studies \(^{(177)}\).

Overall, these results indicate that barley cultivars differ in their physico-chemical properties, mostly in their viscosity. In the intact barley grain, pearling has no effect on the physic-chemical properties and only a small but significant effect on glycemia; viscosity and β-glucan physiological effectiveness are more important than just the MW in determining glycemia and the GI of the intact grain. However, in the extruded fresh pasta products there was a significant reduction in the physico-chemical properties which translated in higher glycemic
responses and GI. This may have happened due to a significant depolymerization of β-glucan as indicated in the decline of β-glucan ability to induce viscosity \(^{(75)}\).

In conclusion, our results suggest that cultivar differed in their physico-chemical properties and pearling did not have a significant impact on these properties. In contrast, milling and extruding barley grains to create fresh barley pasta resulted in significant decline in the physico-chemical properties an observation that should be considered by both regulating bodies and food manufacturing industry when developing barley based products. MW, viscosity and β-glucan content and contribution to viscosity appear to be the controlling factors of the ability of barley to attenuate glycemic responses and the GI.
Table 1. Average values for Starch characteristics of barley cultivars and fractions (g/serving)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fraction*</th>
<th>Serving</th>
<th>Total Starch (g)</th>
<th>Amylose (g)</th>
<th>Total Fiber (g)</th>
<th>β-glucan (g)</th>
<th>RAG (g)</th>
<th>RDS (g)</th>
<th>SDS (g)</th>
<th>RS (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC-Parkhill</td>
<td>WG</td>
<td>87.6</td>
<td>49.3</td>
<td>11.8</td>
<td>16.0</td>
<td>3.8</td>
<td>18.6</td>
<td>14.4</td>
<td>19.6</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>WP</td>
<td>76.1</td>
<td>49.7</td>
<td>12.4</td>
<td>10.0</td>
<td>3.4</td>
<td>17.5</td>
<td>15.0</td>
<td>19.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Celebrity</td>
<td>WG</td>
<td>92.2</td>
<td>49.2</td>
<td>12.0</td>
<td>20.5</td>
<td>5.5</td>
<td>23.0</td>
<td>18.1</td>
<td>29.1</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>WP</td>
<td>80.2</td>
<td>49.6</td>
<td>12.8</td>
<td>13.3</td>
<td>5.0</td>
<td>20.7</td>
<td>17.7</td>
<td>24.8</td>
<td>0.3</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WG</td>
<td>102.2</td>
<td>48.7</td>
<td>1.3</td>
<td>18.9</td>
<td>11.4</td>
<td>37.9</td>
<td>30.6</td>
<td>35.7</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>WP</td>
<td>86.9</td>
<td>49.3</td>
<td>1.4</td>
<td>11.9</td>
<td>10.6</td>
<td>29.0</td>
<td>24.2</td>
<td>26.0</td>
<td>0.1</td>
</tr>
</tbody>
</table>

*WG = whole grain and WP = white pearled. \( n = 3 \). RAG = Rapidly available glucose. RDS = Rapidly digested starch. SDS = slowly digested starch. RS = Resistant Starch.
Table 2. Physico-chemical Characteristics of Cooked Barley Kernels

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fraction</th>
<th>Food Form</th>
<th>β-glucan content (% db)</th>
<th>MW of β-glucan (g/mol×10^3)</th>
<th>% Soluble β-glucan</th>
<th>Viscosity of β-glucan slurry (mPa•sec)</th>
<th>Viscosity of β-glucan extract (mPa•sec)</th>
<th>% Viscosity of β-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celebrity</td>
<td>WG</td>
<td>Boiled Kernel</td>
<td>5.9 ± 0.14^a</td>
<td>2310 ± 600</td>
<td>36.99 ± 2.9^a</td>
<td>2467 ± 46^ab</td>
<td>1460.33 ± 30^ab</td>
<td>59%</td>
</tr>
<tr>
<td>Celebrity</td>
<td>WP</td>
<td>Boiled Kernel</td>
<td>6.1 ± 0.13^a</td>
<td>1870 ± 200</td>
<td>40.33 ± 0.5^a</td>
<td>2331 ± 184^ab</td>
<td>393.00 ± 14^ab</td>
<td>17%</td>
</tr>
<tr>
<td>AC-Parkhill</td>
<td>WG</td>
<td>Boiled Kernel</td>
<td>4.2 ± 0.04^a</td>
<td>1460±320</td>
<td>47.00 ± 0.7^a</td>
<td>826 ± 83^a</td>
<td>55.10 ± 2.0^a</td>
<td>7%</td>
</tr>
<tr>
<td>AC-Parkhill</td>
<td>WP</td>
<td>Boiled Kernel</td>
<td>4.4 ± 0.13^a</td>
<td>1640±656</td>
<td>42.88 ± 0.7^a</td>
<td>2954 ± 410^a</td>
<td>1662.67 ± 43^a</td>
<td>56%</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WG</td>
<td>Boiled Kernel</td>
<td>9.5 ± 1.6^b</td>
<td>2290 ± 105</td>
<td>78.21 ± 1.8^b</td>
<td>1960 ± 92^b</td>
<td>882.00 ± 105^b</td>
<td>45%</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WP</td>
<td>Boiled Kernel</td>
<td>11.1 ± 0.4^b</td>
<td>1960 ± 140</td>
<td>81.01 ± 0.8^b</td>
<td>3906 ± 102^b</td>
<td>1748.67 ± 239^b</td>
<td>45%</td>
</tr>
</tbody>
</table>

n = 3
^c Significant main effect of cultivar, P < 0.05, means with different superscripts are significantly different. There was no significant main effect of pearling.
^a WG: Whole grain, WP: White Pearled
^b Mean ± SD
^c Percent dry basis
^d MW = Molecular weight
^e Viscosity as measured by Rapid Visco Analyzer (RVA)
^f Measured by flow-injection analysis (FIA)
Table 3. Physico-chemical Characteristics of Cooked Barley Pasta*  

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fraction</th>
<th>Food Form</th>
<th>β-glucan content (% db)*</th>
<th>MW of β-glucan (g/mol×10³)§</th>
<th>% Soluble β-glucan</th>
<th>Viscosity of β-glucan slurry (mPa•sec)€</th>
<th>Viscosity of β-glucan extract (mPa•sec)*</th>
<th>% Extract Viscosity of β-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celebrity</td>
<td>WG</td>
<td>Pasta</td>
<td>6.1 ± 0.2^a</td>
<td>1130 ± 331^ab</td>
<td>34.47 ± 0.1^a</td>
<td>840 ± 80^b</td>
<td>34.70 ± 5.0^a</td>
<td>4%</td>
</tr>
<tr>
<td>Celebrity</td>
<td>WP</td>
<td>Pasta</td>
<td>6.2 ± 0.5^a</td>
<td>810 ± 865^ab</td>
<td>46.77 ± 0.6^a</td>
<td>230 ± 18^ab</td>
<td>26.13 ± 4.0^a</td>
<td>11%</td>
</tr>
<tr>
<td>AC-Parkhill</td>
<td>WG</td>
<td>Pasta</td>
<td>4.0 ± 0.01^a</td>
<td>250 ± 280^a</td>
<td>45.86 ± 0.9^ab</td>
<td>372 ± 45^a</td>
<td>23.63 ± 3.0^a</td>
<td>6%</td>
</tr>
<tr>
<td>AC-Parkhill</td>
<td>WP</td>
<td>Pasta</td>
<td>4.2 ± 0.2^2</td>
<td>490 ± 101^a</td>
<td>54.60 ± 0.5^ab</td>
<td>223 ± 35^a</td>
<td>21.73 ± 1.0^a</td>
<td>10%</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WG</td>
<td>Pasta</td>
<td>11.30 ± 0.89^b</td>
<td>1240 ± 131^b</td>
<td>58.74 ± 0.3^b</td>
<td>947 ± 19^b</td>
<td>476.3 ± 3 14^b</td>
<td>50%</td>
</tr>
<tr>
<td>CDC Fibar</td>
<td>WP</td>
<td>Pasta</td>
<td>11.07 ± 0.5^b</td>
<td>1100 ± 250^b</td>
<td>63.50 ± 0.9^b</td>
<td>470 ± 198^b</td>
<td>114.00 ± 5.0^b</td>
<td>24%</td>
</tr>
</tbody>
</table>

n = 3  

* Significant main effect of cultivar, P < 0.05, means with different superscripts are significantly different. There was no significant main effect of pearling.  
^ WG: Whole grain, WP: White Pearled  
^ Mean ± SD  
§ Percent dry basis  
§ MW = Molecular weight  
€ Viscosity as measured by Rapid Visco Analyze  
* Measured by flow-injection analysis (FIA)
Figure 1. Correlation between the glycemic index (GI) and log_{10} MW (A), log_{10} Viscosity (B), log_{10} Extract Viscosity (C), log_{10} % β-glucan contribution (D), Peak blood glucose rise (PBGR) (E) and log_{10} solubility (F) for barley products. Values are means ± SD, n = 10. Viscosity = slurry viscosity of starch and β-glucan.; Extract viscosity = only β-glucan viscosity. Filled circles, whole-grain barley; open circles, pearled barley.
Figure 2. Correlation between the Peak blood glucose rise (PBGR) and log 10 MW (A), log 10 Viscosity (B), log 10 Extract Viscosity (C) and log 10 % β-glucan contribution (D), for barley products. Values are means ± SD, n = 10. Viscosity = slurry viscosity of starch and β-glucan; Extract viscosity = only β-glucan viscosity. Filled circles, whole-grain barley; open circles, pearled barley.
7. *Overall Discussion*
7.1. Discussion

The results of these experiments suggest that differences in chemical composition between cultivars have a significant effect on glycemia and the GI but not on subjective satiety. The significance of variations in chemical composition indicates that when barley is consumed a synergistic effect of the nature of starch and soluble fibre, mainly β-glucan, occur. This is of interest because it challenges the previous notion that only the nature of starch is the determining factor in starch digestibility and its physiological effects. Amylose (unbranched α-(1-4) linked molecules) and amylopectin (branched α–(1-4) and α–(1-6) linkages) are the two main components of starch. The branched structure of amylopectin is more susceptible to hydrolysis than the nearly linear structure of amylose which increases the rate of digestion (17). Therefore, it is assumed that amylose-to-amylopectin ratio influences the rate of starch digestion and in turn dictates the concentration of RDS and SDS (18). Accordingly, it would be predicted that a barley cultivar with a lower percentage of amylose would have a higher GI but this was not the case with CDC Fibar which had the lowest content of amylose and the lowest GI of all the tested cultivars. One reason why CDC Fibar had a low GI, despite low amylose content, may be because of its high content of β-glucan. However, evidence from the first study (Chapter 4) does not support a strong role of β-glucan in determining the GI of cooked whole and pearled barley kernels because GI was more strongly related to total fiber content of barley rather than just β-glucan content (Chapter 4, Figure 1). It’s more likely that the GI of barley is influenced by several competing factors including starch and the physico-chemical properties of soluble fiber and most importantly their interactions.

Indeed, this is further supported by results from the in vitro starch digestibility analysis. RDS content varied between cultivars with CDC Fibar and CDC Rattan containing significantly
higher amounts of RDS (26.9-33.4%) compared to other barleys (14.9-26.2%). Similarly, both cultivars had significantly higher starch digestion index compared to other cultivars (82), yet they had the lowest GI values. Furthermore, an examination of the physico-chemical properties of selected cultivars and their fractions (CDC-Fibar, Celebrity and AC-Parkhill) provided further insight to the synergistic effect of the nature of starch and the physico-chemical properties of β-glucan. For example, CDC-Fibar contained high amount of RDS and high starch digestion index but it also contained the highest content of: β-glucan, solubility, viscosity and β-glucan contribution to viscosity regardless of level of pearling compared to Celebrity and AC-Parkhill (Chapter 4, Table 2). This suggests that CDC-Fibar had the most effective type of β-glucan. Whether this is related to only the amount of β-glucan is not known, but our results do not suggest such an observation. Taken together these results indicate the superiority of the physico-chemical properties of β-glucan over the nature of starch as determinants of glycemic response, but together they have a synergetic effect that enhances the physiological effects of consuming barley.

Correlation results from both chapter 4 and 6 indicate that β-glucan content was not associated with the AUC nor the GI and only total fiber was negatively correlated with the GI of the barley cultivars (Chapter 4, Figure 1, r = -0.81, n = 9, P = 0.008). In this context it is of interest that pearling reduced the total fiber content of barley to a greater extent than that of the β-glucan (82). This suggests that the starch in the outer layers of barley kernels which are removed by pearling is protected by fiber other than β-glucan to a greater extent and has a lower GI than the starch in the center of the barley kernels. This might explain why the WP fraction, which removes 25-30% of the starch from barley, increases the GI of intact barley (Chapter 4, Table 2).
Our results suggest that the matrix structure of barley is a more important determinant of its GI than its content of RDS, SDS, RS or β-glucan. The disruption of the matrix structure of barley by grinding and extruding barley to create fresh 100% barley pasta increased the GI significantly compared to their native intact kernels. The barley pastas had unexpectedly relatively high GI values since pasta is considered to have a low GI. However, we believe our pasta had a high GI because we produced wet or fresh pastas, whereas pasta is usually available as dry pasta. The drying process could harden the matrix structure and make it less accessible to enzymes, and also inactivate indigenous β-glucan degrading enzymes, while wet pasta is more readily available or accessible to indigenous and external enzymes. This was supported by the high GI of the control wet pasta made from semolina flour compared with dry semolina pasta (GI ≈ 41) (90). It is of interest that pasta made from pearled barley had a lower GI than wholegrain barley pasta, whereas pearling increased the GI of intact barley kernels.

The disruption of the matrix structure of barley grains resulted in a significant reduction in the physico-chemical properties of β-glucan (Chapter 4, Table 3). Consequently, this disruption resulted in a severe loss of barley’s β-glucan bioactivity and physiological effectiveness which is its ability to develop viscosity in the digestive tract. Increasing viscosity of the meal bolus in the stomach is now recognized as a mechanism responsible for the positive health effects of consuming β-glucan rich foods. Intestinal viscosity is thought to be responsible for reducing the interactions of food with digestive enzymes and delaying gastric emptying. Increasing the viscosity has also been shown to retard the absorption of glucose and slow the rate of starch digestion in in vitro digestion model studies.

In previous studies it has been suggested that MW of β-glucan is the determining factor in the health benefits associated with consuming β-glucan rich products (177). Our results show
that all three cultivars had relatively similar MW (Chapter 4, Table 2 and 3) but they differed significantly in their viscosity and the ability of β-glucan to develop viscosity (Chapter 4, Table 2 and 3). This suggests that for MW at a maximum value it is the content of β-glucan that determines the development of viscosity.

This thesis has a number of limitations, including lack of other postprandial measurements such as insulin and gut hormones. Previous studies have shown that barley food products with a low GI also elicit low insulin responses and may have an effect on a number of gut hormones (102, 142, 181). Although we only studied normal subjects, the values can be generalized to other populations because previous studies suggest that the GI values of starchy foods are similar in normal, hyperinsulinemic and diabetic subjects (163). In measuring subjective satiety we did not use the gold standard measure, Visual Analogue Scales or energy intake, due to feasibility and time constraints. Moreover, we did not have statistical power to measure satiety with great precision. Yet, satiety rating scales and the satiety index are two methods that have been previously used by other authors and have yielded reliable and reproducible results (182-185).

Future directions include investigating the relationship between barley and inflammatory responses. It has been hypothesized that low-GI foods can reduce inflammatory responses acutely. Whether this is related to their chemical composition (i.e. nature of starch, fibre content) and GI is not completely explored. Barley is also a rich source of antioxidants and whether they have an acute effect on inflammation is not known and if the effect on inflammation is separate from their GI or is a synergistic one is also not known. The impact of processing on the antioxidants capacity of barley in reducing inflammation has not been completely explored.
Barley fractions with different levels of antioxidants, starch and fiber can be used to compare the contribution of each component separately and assess if an interaction between them exists. Furthermore, the relationship between viscosity and satiety can be further explored. In this study we did not assess the physico-chemical properties of all the cultivars studied which limited our ability to assess the relationship between viscosity, MW and satiety.
8. Conclusion
This thesis has set out to answer the following research questions:

This thesis will attempt to answer the following questions:

1. What is the significance of differences in chemical composition, total fibre and β-glucan content on glycemic response, GI and subjective satiety?
2. What is the effect of food form on glycemic response, GI and subjective satiety?
3. What is the magnitude and impact of processing and food form on the physico-chemical properties of barley (MW, solubility and viscosity) ?
4. What is the contribution of the physico-chemical properties with respect to glycemia and the GI?

Our results indicate that the chemical composition has significant importance with regard to postprandial responses, in particular, the interaction between the starch and β-glucan and their combined effect on the AUC and the GI. However, this relationship does not appear to have an effect on subjective satiety. Food form is a significant factor in affecting the glycemic response and the GI of cooked barley and barley products. Altering the natural intact structure of barley kernel and consequently the fibre matrix results in structural changes that reduces the bioactivity of β-glucan and its ability to induce viscosity the digesta. In turn, this affects the GI of the food, an inherent characteristic of carbohydrates rich foods, by allowing degrading enzyme easier accessibility. Nonetheless, food form may have a differential effect on satiety than that on glycemia and the GI. Our results indicate that the postprandial effects associated with consuming the intact whole grain can be relatively maintained with different levels of pearling
but not with severe food form alteration. Pearling is a mild process that does not affect the physico-chemical properties significantly. In contrast milling and extruding the grain where it changes the food form significantly had a deleterious impact on the bioactivity of the \( \beta \)-glucan. In particular, milling and extruding barley kernels to create a wet pasta reduced the MW, solubility and viscosity significantly.

In conclusion, this thesis was able to answer a number of important questions regarding the association between whole grains and glycemia. We used barley as a surrogate measure due to its increasing demand and the development of a number of specialty cultivars that are produced for human consumption in Canada. The trend of barley end use seems to take the direction of a functional food rather than being consumed as a whole grain. Our results show that the intact kernel is superior in producing positive physiological responses on glycemia and maintaining a low GI, a more significant measure of CHO quality. The results of this thesis are consistent with those of previous studies which suggest that whole grains, that can be processed into a variety of different forms, do not necessarily have a low GI. Therefore, there is a need for improved methods of how whole grains are classified. Selecting a low GI barley cultivar can help not only blunt high postprandial blood glucose levels but also reduce the overall glycemic load of a meal, a dietary maneuver that could produce enhanced public health benefits. Our Results also underscore the significance of the physico-chemical characteristics of foods; \( \beta \)-glucan ability to contribute to viscosity and MW should be highly considered in future definition of whole grains and product development.


123. Shikany JM, Flood AP, Kitahara CM et al. (2011) Dietary carbohydrate, glycemic index, glycemic load, and risk of prostate cancer in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) cohort. Cancer Causes and Control 22(7), 995-1002.


135. Liu AG, Most MM, Brashear MM et al. (2012) Reducing the glycemic index or carbohydrate content of mixed meals reduces postprandial glycemia and insulinemia over the entire day but does not affect satiety. Diabetes Care 35(8), 1633-1637.


10. **Appendices**
10.1 Consent Form

SUBJECT INFORMATION AND CONSENT FORM

**TITLE:** EFFECTS OF VARIETY AND PEARLING ON THE GLYCAEMIC IMPACT OF BARLEY (GIL-8037)

**SPONSORS:**
- Agriculture and Agri-Food Canada
- Ontario Ministry of Agriculture, Food and Rural Affairs
- Out and Barley Council of Ontario, PO Box 175, Gormley, ON, L3M 4C3
- Gilbertson & Page (Canada) Inc., 501 Dickson Dr, Fergus, ON, N1M 2W8
- Gain Process Enterprises Ltd., 115 Commander Blvd, Scarborough, ON, M1S 3M7
- Cibi Seeds/Wintercon Farms, 265 Katherine St, South R.R. #2, West Montrose, ON, N0B 2V0

**INVESTIGATORS:**
- Thomas MS Wolfer, PhD, DM,
  Professor, Department of Nutritional Sciences
  University of Toronto, Toronto, ON M5S 3E2
tel: 416-978-5556 e-mail: thomas.wolfer@utoronto.ca

- Ahmed Aldughassi, MSc
  Graduate student, Department of Nutritional Sciences
  University of Toronto, Toronto, ON M5S 3E2
tel: 416-978-0548 e-mail: ahmed.alduhghassi@utoronto.ca

This study is part of the research being carried out for a PhD Degree.

**SITES:**
- 36 Lombard Street, Suite 100
  Toronto, Ontario M5C 2X3

This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand.

F 750-11

Approved by
U of T Ethics Board,
Original Approval May 8, 2007
Amendment Approval July 22, 2008
Protocol Reference #20145, #22750
INTRODUCTION/PURPOSE

Foods such as bread, rice, pasta, cereals and legumes contain starch (complex carbohydrate). The degree to which these foods raise blood glucose (blood sugar) is classified using the glycaemic index. Whole grain foods and foods containing a high amount of dietary fibre tend to have a low glycaemic index and may help reduce the risk of obesity, diabetes and coronary heart disease.

Barley, a bland tasting grain, is recognized as a low glycaemic index cereal which may offer significant benefits to people with diabetes, to those trying to control their body weight and for the general population. Barley is rich in dietary fibre, starch and nutrients such as vitamins and minerals. Pearled barley is a common food ingredient, however, the effects of pearling (a grinding process which removes the husk and outer layers of the seed) on the blood glucose response of barley is unknown. Also, there are many varieties of barley which differ in the amounts and types of starch and dietary fibre they contain.

The purpose of this study is to see if varieties of barley containing different types of starch and amounts of fibre have different effects on blood glucose responses, and to see how the pearling process affects the blood glucose response. This study is part of a larger research project to develop tasty and healthy food products made from barley.

PROCEDURES

You will be asked to come to 36 Lombard Street, Suite 100 between 7 a.m. and 9:30 a.m. after a 10 to 14 hr. overnight fast on 3 separate mornings. You will be asked to come one to two times per week over a period of 5-4 weeks. Each test will last about 24 hrs and requires 7 blood samples to be taken by finger-prick. Each time you come, you will be weighed and will write down the time of your last meal and any unusual activities or circumstances (e.g., illness) of the previous day. You will then be given a fasting blood sample (2-3 drops) and be given a test meal consisting of a bowl of cooked barley or 3 slices of white bread plus a drink of 1 or 2 cups of water, tea or coffee with milk and artificial sweetener if desired. The drink you choose will remain the same for all the other tests.

There will be 4 different types of barley test meal, and 1 remaining pasta: varieties of barley will be used which differ in the amount and types of starch and dietary fibre they contain. Each variety will be either whole grain or pearled. You will receive about 1/2 cup of cooked barley pasta (boiled in water). The white bread will be baked in an automatic bread maker. The responses of the barley meals will be compared to that of white bread. The test of bread will be done 3 times and the average value used to reduce the variation of the results.

The test meal should be consumed within 10 minutes or as fast as you can if it takes longer than 10 minutes. Further blood samples (2-3 drops each) will be taken by finger-prick at 15, 30, 45, 60, 90 and 120 minutes after starting to eat (7 blood samples in total each day). You
will also fill out a questionnaire indicating how palatable you considered the test meal and how
hungry or full you feel just before each blood sample. You will be asked to remain seated
throughout the test. At the end of the test you will be offered a snack and be free to leave.

RISKS

Side effects from the test meals may cause nausea, vomiting, cramping, diarrhea, or
bloating. The expected incidence of these and other types of side effects is rare. Barley is high
in dietary fiber and you may experience more flatulence than normal on the days when you have
the barley meals.

The risks of blood sampling include dizziness, fainting, discomfort, redness, swelling,
bruising, and rarely, infection at the site where the needle punctures the skin. The needle prick
usually causes some discomfort, but the risks of any of the other side effects happening are rare.
Subjects are advised to prick their fingers at the sides to minimize the discomfort.

Accidentally sticking someone else with your used needle or being stuck with someone
else’s used needle could transmit AIDS, hepatitis or other potentially serious infections. The risk
of this is minimized by providing you with your own lancet device which is not to be shared with
anyone else, using a new needle for each finger-prick, and having you prick your own finger
and removing your own used needle immediately placing it into the disposal container provided.
Research staff can assist subjects with blood samples if required. The lancet devices are
designed in such a way that the needle is enclosed within the body of the device and is only
exposed momentarily when the device is cocked and “fired”. Subjects who do not follow the
instructions of the research staff about the safe handling of needles or who behave in ways which
increases the risk of harm to themselves or others may not be allowed to participate any more.

There may be risks or side effects which are unknown at this time.

BENEFITS

You will not benefit directly from participating in this study.

PAYMENT FOR PARTICIPATION

Your results are not useful unless you complete all the tests requested. You will be paid
$29.00 plus the cost of 2 TTC tokens for each test completed. You may be asked to repeat tests if
the results are poor. As long as this is not a result of your failure to follow instructions, you will
be paid $29.00 plus the cost of 2 TTC tokens for each test repeated.

ALTERNATIVE TREATMENT

This is not a treatment study. You do not have to participate in this study.

VOLUNTARY PARTICIPATION/WITHDRAWAL

F 750-11
Approved by
U of T Ethics Board,
Original Approval May 8, 2007
Amendment Approval July 22, 2008
Protocol Reference #2014R, #22750
Your participation in this study is voluntary. You may decide not to participate or you may withdraw from the study at any time.

The investigator may stop your participation in these tests if you do not follow the safety guidelines, if you do not follow the instructions of the study doctor or study staff, or if your results are not able to be used.

CONFIDENTIALITY

The information gathered from you during this study will be kept confidential unless required by law. In addition, the information from this study may be examined by the Ethics Review Board of the University of Toronto. The results of this research study may be presented at meetings or in publications, however, your identity will not be disclosed in these presentations.

CONFLICTS OF INTEREST

This study is being carried out at GILaboratories which is a privately owned company of which Dr. Wolfever is president and part-owner. GILabs is involved because space and equipment are not available at the University of Toronto or elsewhere. GILabs will recover only its costs for performing the study. GILabs will not own the results and Dr. Wolfever will not be paid.

QUESTIONS

If you have any questions concerning your participation in this study, or if at any time you feel you have experienced a research-related injury you may contact the study doctor, Dr. Thomas M.S. Wolfever at (416) 978-5556 during business hours (Monday to Friday 9:00-5:00) or e-mail him at thomas.wolfever@utoronto.ca.

If you have questions about your rights as a research subject, you may contact Jenny Peto, Director, Ethics Review Office, University of Toronto at (416) 946-3389.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

If you agree to participate in this study, you will receive a signed and dated copy of this consent form for your records.

F 750-11

Approved by
U of T Ethics Board,
Original Approval May 8, 2007
Amendment Approval July 22, 2008
Protocol Reference #20145, #22750
CONSENT

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without prejudice. By signing this consent form I have not waived any of the legal rights which I otherwise would have as a participant in a research study. As well, the potential risks, harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study.

I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties. I know that I may ask now, or in the future, any questions I have about the study or the research procedures. I have been assured that records relating to me and my care will be kept confidential and that no information will be released or printed that would disclose personal identity without my permission unless required by law. I have been given sufficient time to read and understand the above information.

I hereby consent to participate, and I will be given a signed copy of this consent form.

Subject name: ______________________________

Subject signature: __________________________ Date: ________________

Name and Position of Person Conducting Informed Consent Decision:

Name: __________________________ Position: __________________________

Signature: __________________________ Date: ________________
10.2 Screening form

<table>
<thead>
<tr>
<th>Glycemic Index Laboratories</th>
<th>20 Victoria Street, 3rd Floor</th>
<th>Tel 416.861.8505</th>
<th>Fax 416.861.7694</th>
<th><a href="http://www.gllabs.com">www.gllabs.com</a></th>
<th>SUBJECT</th>
<th>SCREENING</th>
<th>FORM</th>
<th>Page 1 of 3</th>
</tr>
</thead>
<tbody>
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YES NO

☐ ☐ 1. Subject is between 18 and 75 years of age, inclusively.

☐ ☐ 2. Subject is a male or a non-pregnant female at least 6 weeks postpartum and non-lactating.

☐ ☐ 3. Subject does not have diabetes mellitus or glucose intolerance.

☐ ☐ 4. Subject is free from active metabolic or gastrointestinal diseases that may interfere with nutrient absorption, distribution, metabolism, excretion and have no known food allergies.

☐ ☐ 5. Subject has not had recent infection (requiring medication or hospitalization), surgery, corticosteroid treatment in the last 3 months or antibiotics in the last 3 weeks.

☐ ☐ 6. Subject is not taking daily medications (e.g., Acetaminophen, salicylates, diuretics, etc.) that would interfere with nutrient absorption, metabolism, excretion or gastric motility.

☐ ☐ 7. Subject does not have AIDS, hepatitis, a heart problem or any other medical condition which might make participation dangerous or affect the results.

☐ ☐ 8. Subject or subject's legally authorized representative has voluntarily signed and dated an informed consent form prior to any participation in the study.
DEMOGRAPHIC INFORMATION

Date of Birth:  

Day:  
Month:  
Year:  
Age:  

Sex:  
1) Male  
2) Female

Ethnicity:  
1) Caucasian  
2) Black (e.g. African, Haitian, Jamaican, Somali)  
3) Aboriginal  
4) South Asian (e.g. East Indian, Pakistani, Punjabi, Sri Lankan)  
5) Arab/West Asian (e.g. Armenian, Egyptian, Iranian, Lebanese, Moroccan)  
6) Filipino  
7) South East Asian (e.g. Cambodian, Indonesian, Vietnamese)  
8) Latin American  
9) Chinese  
10) Japanese  
11) Korean  
12) Other: __________________________

GIL:  
Subject Number:  
Subject Initials:  
SUBJECT SCREENING FORM

20 Victoria Street, 3rd Floor
Toronto ON Canada M5C 2N8
Tel 416-861-0806
Fax 416-861-0649
www.glab.com
<table>
<thead>
<tr>
<th>Glycemic Index Laboratories</th>
<th>20 Victoria Street, 5th Floor Toronto ON Canada M5C 2N8 Tel 416.861.0505 Fax 416.861.0649 <a href="http://www.gilabs.com">www.gilabs.com</a></th>
<th>SUBJECT SCREENING FORM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject Initials</td>
<td>Subject Number</td>
<td>GIL #</td>
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</tr>
<tr>
<td>Height: [ ] [ ] [ ] [ ] cm</td>
<td>Weight: [ ] [ ] [ ] [ ] kg</td>
<td>BMI: [ ] [ ] [ ]</td>
</tr>
</tbody>
</table>

Medications: __________________________________________________________
__________________________________________________________
__________________________________________________________
__________________________________________________________

Screening Results  □ 1) Pass  □ 2) Failure

Date History Taken: ____________________________

Information Obtained By: ________________________

---

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10.3 Blood Glucose Record

Subject Initials: [ ] [ ] Subject ID No: [ ] [ ] Date: [ ] [ ] [ ] [ ] [ ]

Weight: _________ kg Study: ______________

Test Meal: _____________________________

Beverage: _________ ml Type: [ ] Coffee [ ] Tea [ ] Water
Additions: [ ] none [ ] 30 ml milk [ ] artificial sweetener

Time of starting to eat: ________ am Time taken to eat: ________ minutes

Please make a mark along the line to indicate meal palatability:

Very unpalatable ________________ Very Palatable

Blood Samples taken on time - write in time if not

<table>
<thead>
<tr>
<th>5&quot;</th>
<th>0&quot;</th>
<th>15&quot;</th>
<th>30&quot;</th>
<th>45&quot;</th>
<th>60&quot;</th>
<th>90&quot;</th>
<th>120&quot;</th>
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Blood Glucose Results (mmol/L)

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<tr>
<th>-5&quot;</th>
<th>0&quot; (1)</th>
<th>0&quot; (2)</th>
<th>15&quot;</th>
<th>30&quot;</th>
<th>45&quot;</th>
<th>60&quot;</th>
<th>90&quot;</th>
<th>120&quot;</th>
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</table>

Was alcohol consumed the previous evening? [ ] Yes [ ] No

If yes, describe: ____________________________

Were there any changes to your medications since your last visit? [ ] Yes [ ] No

If yes, describe: ____________________________

Time of last meal: ______________

Composition of last meal:

Any unusual occurrences yesterday (e.g. activity, food, illness):

FOR OFFICE USE ONLY

Staff Initials: ____________________________ YSI Results checked: ______________

C:\Users\Ahmed\Downloads\F 730-03 Blood Glucose Test Record %28ae%29.doc F 730-03
### 10.4 Satiety Score

**SATIETY SCORE SHEET**

<table>
<thead>
<tr>
<th>Time</th>
<th>Extremely Hungry</th>
<th>Hungry</th>
<th>Semi Hungry</th>
<th>No Particular Feeling</th>
<th>Semi Satisfied</th>
<th>Satisfied</th>
<th>Extremely Full</th>
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</thead>
<tbody>
<tr>
<td>Fasting</td>
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<td>15 min</td>
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<td>120 min</td>
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</tbody>
</table>

Initials: ___________________ Subject ID No.: ___________________ Date: _______________

Test Meal: ___________________ Study: _______________

Just before each blood sample:
makes one vertical mark along the line to indicate how hungry/full you feel.
### 10.5 Barley and Semolina Pasta Recipes

<table>
<thead>
<tr>
<th>CELEBRITY WG</th>
<th>CELEBRITY WP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients</strong></td>
<td><strong>Ingredients</strong></td>
</tr>
<tr>
<td>100 g F-1 of Celebrity</td>
<td>100 g F-4 of Celebrity Barley</td>
</tr>
<tr>
<td>1g salt</td>
<td>1g salt</td>
</tr>
<tr>
<td>1 g Xanthan</td>
<td>1 g Xanthan</td>
</tr>
<tr>
<td><strong>70g water</strong></td>
<td><strong>60 g water</strong></td>
</tr>
<tr>
<td><strong>86 µL Annato</strong></td>
<td><strong>81.0 µL Annato</strong></td>
</tr>
<tr>
<td><strong>Cooking</strong></td>
<td><strong>Cooking</strong></td>
</tr>
<tr>
<td>2 min dry Mixing</td>
<td>2 min dry Mixing</td>
</tr>
<tr>
<td>13 min wet mixing</td>
<td>13 min wet mixing</td>
</tr>
<tr>
<td>5 min boiling</td>
<td>5 min boiling</td>
</tr>
<tr>
<td><strong>Serving Size: 224.4g</strong></td>
<td><strong>Serving Size: 207.7g</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>AC-PARKHILL WG</th>
<th>AC-PARKHILL WP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ingredients</strong></td>
<td><strong>Ingredients</strong></td>
</tr>
<tr>
<td>100 g F-1 of AC-PARKHILL</td>
<td>100 g F-4 of AC-PARKHILL</td>
</tr>
<tr>
<td>1g salt</td>
<td>1g salt</td>
</tr>
<tr>
<td>1 g Xanthan</td>
<td>1 g Xanthan</td>
</tr>
<tr>
<td><strong>65 g water</strong></td>
<td><strong>60 g water</strong></td>
</tr>
<tr>
<td><strong>83.5 µL Annato</strong></td>
<td><strong>81 µL Annato</strong></td>
</tr>
<tr>
<td><strong>Cooking</strong></td>
<td><strong>Cooking</strong></td>
</tr>
<tr>
<td>2 min dry Mixing</td>
<td>2 min dry Mixing</td>
</tr>
<tr>
<td>13 min wet mixing</td>
<td>13 min wet mixing</td>
</tr>
<tr>
<td>5 min boiling</td>
<td>5 min boiling</td>
</tr>
<tr>
<td><strong>Serving Size: 199.1g</strong></td>
<td><strong>Serving Size: 182.6</strong></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>SEMOLINA</th>
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</tr>
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<tbody>
<tr>
<td><strong>Ingredients</strong></td>
<td><strong>Ingredients</strong></td>
</tr>
<tr>
<td>100 g semolina</td>
<td>100 g semolina</td>
</tr>
<tr>
<td>1 g salt</td>
<td>1 g salt</td>
</tr>
<tr>
<td><strong>35 g water</strong></td>
<td><strong>35 g water</strong></td>
</tr>
<tr>
<td><strong>Cooking</strong></td>
<td><strong>Cooking</strong></td>
</tr>
<tr>
<td><strong>2 min dry mixing</strong></td>
<td><strong>2 min dry mixing</strong></td>
</tr>
<tr>
<td><strong>3 min wet mixing</strong></td>
<td><strong>3 min wet mixing</strong></td>
</tr>
<tr>
<td><strong>20 min boiling</strong></td>
<td><strong>20 min boiling</strong></td>
</tr>
<tr>
<td><strong>Serving Size: 205.5g</strong></td>
<td><strong>Serving Size: 205.5g</strong></td>
</tr>
</tbody>
</table>

*Only 100 g of pasta can be made in each batch using the Pstamatic Machine*
10.6 In vitro digestion

IN VITRO DIGESTION AT 37 °C

This digestion procedure suits for cereals and processed products. Soluble fibre (beta-glucan) is extracted by digestible enzymes.

Reagents:
Buffer: Sodium Phosphate
20 mM NaH₂PO₄ + 10 mM NaCl
2.76 g NaH₂PO₄·H₂O
900 ml H₂O
Adjust the pH to 6.9 and fill into a 1 L
Add 0.58 g NaCl

Amylase: Alpha-amylase EC:3.2.1.1, from human saliva, 46 U/mg solid (Sigma A-1031)
5 mg/ml in 3.2 mM CaCl₂ solution

Pepsin: Pepsin A EC:3.4.23.1 2260 U/mg solid from porcine stomach mucosa
(Sigma P-7012)
0.5 mg/ml in 0.9% NaCl-solution
0.48 g NaCl
50 ml H₂O
0.055 g Pepsin

Pancreatin: Pancreatin from porcine pancreas EEC:232-468-9 (Sigma P7545)
0.5 mg/ml in buffer
Dissolve in 37 °C about 20 min.

Store enzyme and buffer at + 4 °C.

Sample preparation:
Dried or fresh sample, dry sample is grinded to 30 mesh.
The water amount of fresh and very watery samples should be taken into account.

For example:
Fresh oatmeal: If the dry matter content is 11 %
⇒ 45 g of sample includes 5 g dry matter and 40 ml water,
therefore only 60 ml buffer is added.

Methods:
Moisture content of the sample materials is measured drying under vacuum 80 °C for 4 h. Moisture content of suspensions and supernatant is measured with sand first in oven and thereafter under vacuum 80 °C 4 h or by freeze drying.

The beta-glucan content of samples material are analysed according to the Approved enzymatic method 32-23 (AACC 2009). For this analysis, processed samples are ethanol treated by 50% (v/v) aqueous ethanol to remove free sugars and to reduce the levels of fats. The beta-glucan content in vitro digestion extract is measured with FIA