Interaction Between the Effects of Preparation Method and Variety on the Glycemic Index of Novel Potato Varieties

by

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

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ABSTRACT

As part of a project to see whether potatoes with a low glycemic-index (GI) could be developed through plant breeding, the GI values of 4 new potato varieties differing in starch structure was determined in 3 studies over 2 years in human subjects. Since cooking and cooling affects starch structure the potatoes were studied both freshly cooked (boiled) and cooled. The first study showed that cooling reduced the GI of two varieties by 40-50% but had no effect in the others (treatment × variety interaction, p=0.024), an effect which was confirmed in study 2. Differences in GI were readily explained by differences in starch structure or in-vitro digestion rate. Carbohydrate malabsorption increased from 3 to 5% upon cooling, not enough to account for the reduced GI. It is concluded that the effect on GI of cooling cooked potatoes varies in different varieties. Further research is needed to understand the mechanism.
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<td>Agriculture and Agri-food Canada</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>AUC</td>
<td>Area under the curve</td>
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<td>BPN</td>
<td>Bio Potato Network</td>
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<tr>
<td>BV</td>
<td>Biological value</td>
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<td>CHO</td>
<td>Carbohydrate</td>
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<td>CRP</td>
<td>C-reactive protein</td>
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<td>CVD</td>
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<td>FFA</td>
<td>Free fatty acids</td>
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<td>FG</td>
<td>Free glucose</td>
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<td>H₂</td>
<td>Hydrogen gas</td>
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<td>HbA₁c</td>
<td>Glycated hemoglobin</td>
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<td>NEFA</td>
<td>Non esterfied fatty acids</td>
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<td>RAG</td>
<td>Rapidly available glucose</td>
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<tr>
<td>RDA</td>
<td>Recommended Daily Allowance</td>
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<td>RDS</td>
<td>Rapidly digestible starch</td>
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<td>RS</td>
<td>Resistant starch</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>Slowly available glucose</td>
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<td>SDS</td>
<td>Slowly digestible starch</td>
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<td>SEM</td>
<td>Standard error of the mean</td>
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<td>SME</td>
<td>Second meal effect</td>
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Chapter 1

Introduction

The potato (*Solanum tuberosum*) originated approximately 8,000 years ago in South America and first spread to European countries in the 16th century. Since then potatoes have spread worldwide, and are now grown in 160 countries. Canada is the 12th largest potato producer in the world, producing close to 5 million metric tons in 2007 with approximately 160 registered varieties (AAFC, 2007).

Potatoes represent approximately 36% of all fresh and processed vegetables consumed. Fresh potatoes account for 45% of total potatoes consumed, with processed potatoes (i.e. French fries and potato chips) accounting for the remaining 55%. Despite potato popularity, consumption has declined from 76 kg per person in 1994 to 65 kg in 2007 (AAFC, 2007). Canadians are simply preparing fewer fresh potatoes, with a 27% drop in fresh potato consumption between 1997 and 2005. Frozen potato consumption, mainly as French fries has also been decreasing since 2002 (AAFC, 2007).

This decline in potato consumption may be secondary to various dietary trends and negative perceptions concerning its nutritional value. Although one medium potato (150g, boiled with skin intact) contains only 129 kcal, is an excellent source of vitamin C, a good source of potassium, and is virtually sodium and fat free (Health Canada, 2009), potatoes continue to be discouraged by diet books (Steward et al., 2003; Willet, 2001), magazines (Golden, 2002; Willet & Stampfer, 2006) and even scientific articles (Halton et al., 2006; Liu & Willett, 2002; Willet, 2002) due to their purported high Glycemic
Index (GI). High GI diets have been associated with increased risk of diabetes and cardiovascular disease (CVD) and therefore foods with a low GI have been encouraged (Willet et al., 2002; Leeds, 2002). The GI is a method used to classify the blood glucose raising potential of carbohydrate containing foods. It ranks carbohydrates on a scale of 0-100, based on how they raise blood glucose relative to a reference food of glucose or white bread (GI=100) (FAO, 1998; Wolever et al., 1991b).

Although potatoes are perceived as a high GI food, reported GI values vary widely from as low as 23 to as high as 118 (Atkinson et al., 2008). These differences could be due to random experimental error or inconsistencies between methodologies, but may also be explained by factors such as differences in starch structure between varieties, processing methods and harvest dates. This literature review will explore how each of these factors can affect the digestibility of the potato, and ultimately its GI.
Chapter 2

Literature Review

2.1 Potato Nutrition

Newly harvested potatoes contain approximately 80% water and 20% dry matter, with 60-80% of dry matter as carbohydrate predominantly, starch (discussed below) (Camire et al., 2009; USBP, 2008). Potatoes have been perceived to be high in calories and fat compared with other carbohydrate sources such as rice or pasta. This is an incorrect assumption as, potatoes based on 100g weight (boiled, with skin), contain 86 kcal compared to an equal portion of rice (white, long grain, cooked) and pasta (enriched, spaghetti, cooked) at 130 kcal and 141 kcal respectively, and like rice and pasta has negligible fat. Potatoes are also considered a source of dietary fibre, with one medium potato (150 g, boiled) with skin providing approximately 2 grams of fibre (Health Canada, 2009).

Compared to other raw vegetables potatoes are low in overall protein (~2 g per 100 g weight) (Health Canada, 2009). However, the little protein they do contain has excellent biological value (BV), with a BV of 90-100 compared to that of whole eggs (100), or soybeans (84) (Camire et al., 2009). Potatoes are also quite high in certain minerals such as potassium and phosphorus. One medium potato (~150g, boiled with skin) provides 572mg of potassium, more than those foods commonly associated with potassium content, such as bananas (1 medium, 422mg) or oranges (1 medium, 232mg) (Health Canada, 2009). Vitamin C is the predominant vitamin in potatoes, with 1 medium potato
(boiled with skin) containing 18.4 mg, 20% and 25% of the Recommended Daily Allowance (RDA) for adult men and women respectively (IOM, 2000). In addition to vitamins and minerals, potatoes also contain phytochemicals with antioxidant potential, which can vary in amount and composition among varieties (Brown, 2005).

2.1.1 Potato Starch

Starch is the main carbohydrate source in potatoes. It is found in raw potatoes as water insoluble granules (Miles et al., 1985). The two main polysaccharides that can be extracted from these granules are amylace and amylopectin (Gallant et al., 1992). Amylose is a linear un-branched chain containing 500-2,000 glucose residues attached through α1-4 glycosidic bonds. Amylopectin is a highly branched-chain polymer, containing $10^4$-$10^5$ glucose units, linked by both $\alpha$ 1-4 and $\alpha$ 1-6 linkages at the branch points (Gallant et al., 1992; USBP, 2008). Potato starch is typically comprised of 80% amylopectin and 20% amylose (Camire et al., 2009). Potato starch in its native form is resistant to the action of amylolytic digestive enzymes. However, different processing methods, such as cooking and cooling can alter the rate and extent at which glucose is absorbed by the small intestine.

2.1.2 Effects of Cooking and Cooling on Starch

When starches are heated in liquid around temperatures of 60-70°C hydrogen bonds between glucose residues weaken and granular structure is lost. Starch granules take up water and swell resulting in a suspension of porous and swollen granules. The starch is dispersed and is susceptible to hydrolysis by $\alpha$-amylase. This process is termed gelatinization (Miles et al., 1985; Ring et al., 1988). Linear amylose tends to be more
resistant to the process of gelatinization than does amylopectin (Liu et al., 2007). When cooled, the dispersed polymers re-associate with each other by way of hydrogen bonding, forming an irregular structure that is more resistant to digestion. A process known as retrogradation (Englyst et al., 1992a; Miles et al., 1985). Amylopectin is limited in its retrograding capabilities by its branched structure and the polymers that do re-associate are less firmly bound than those of retrograded amylose (Englyst, et al., 1992a). Therefore, upon reheating at approximately 70ºC, crystallized (retrograded) amylopectin is more easily reversed compared to crystallized amylose which requires a higher reheating temperature of 160ºC to reverse the process of retrogradation (Miles et al., 1985; Ring et al., 1988). Based on the effects of processing alone it is evident that starch is hydrolysed to glucose at different rates and extents. These differences have led to the development of measurements and classification systems for carbohydrates based on their digestibility (Englyst et al., 1992a; Englyst et al., 1999; Jenkins et al., 1981; Bond et al., 1972; Englyst et al., 1985).

2.2 Classification and Measurement of Dietary Carbohydrates

The following sections will review both in-vivo and in-vitro methods that have been and are still used to measure and classify carbohydrates based on their absorption in the small intestine.

2.2.1 In Vitro Classification and Measurement

In addition to food processing, the rate and extent of starch digestion also differs due to physicochemical properties of the starch such as amylose:amylopectin ratio and phosphorus content, not yet discussed. Analytical methods have been developed that
characterize dietary carbohydrates with regard to their physicochemical properties and the likely site, rate and extent of digestion in vivo (Englyst et al., 2003).

Englyst et al. (1992a) classified dietary starch based on its susceptibility to α-amylase in vitro. Briefly, rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation with pancreatic amylase at 37°C for 20 min and a 120 min, respectively. Resistant starch is the starch not hydrolysed after 120 min incubation and is calculated by subtracting RDS plus SDS from total starch (TS) (Englyst et al., 1992a). This method provides estimates on the proportion of starch that is likely to be digested rapidly in the small intestine (RDS), slowly (SDS) and the amount likely to pass through to the large intestine (RS).

In more recent years Englyst et al. (1999) modified the Englyst et al. (1992a) method for measuring RDS, SDS and RS in order to measure rapidly available glucose (RAG) and slowly available glucose (SAG) to describe the likely rate and extent of glucose release from starch and sugars in the small intestine. RAG is the amount of glucose measured after incubation of a food sample with digestive enzymes for 20 min (G\textsubscript{20}). Values for RDS can be determined by correcting RAG for free glucose (FG), which is obtained by a separate analysis (RDS= (G\textsubscript{20}-FG) x 0.9). SAG is the amount of glucose measured after 120 min (G\textsubscript{120}) (SAG= G\textsubscript{120}- G\textsubscript{20}). Values for SDS can be calculated by SAG as, SDS= ((G\textsubscript{120}-G\textsubscript{20}) x 0.9). The RS can also be calculated by the fractions measured using the Englyst et al. (1999) method (RS=total glucose – G\textsubscript{120}) x 0.9). Values for the calculated starch fractions are expressed as polysaccharides by the conversion factor 0.9 (Englyst et al., 1999; Englyst et al., 1996b). Depending on the composition of the carbohydrate (i.e. starch or glucose) RAG, SAG and the starch fractions (RDS, SDS and RS) continue to be
measured to classify foods based on their carbohydrate bioavailability. The use of RAG and SAG values instead of the starch fractions however, extends the application of in-vitro analysis to non-starch foods, since RAG and SAG sources are not restricted by the source of glucose (Englyst et al., 1996b).

The physiological relevance of the in-vitro measurements described have been demonstrated through correlation with in-vivo glycemic responses (Englyst et al., 1999; Englyst et al., 2003 Englyst et al., 1996b). So although these methods cannot account for the physiological differences that occur between and within an individual, measurement of RAG, SAG and starch fractions provide a consistent method of characterizing carbohydrate availability for the comparison of different foods (Englyst & Englyst, 2005).

2.2.2 The Glycemic Index

Unlike the in-vitro methods described above, the GI takes a physiological approach in classifying foods based of their ability to raise blood glucose on a scale from 0-100. It is defined as the incremental area under the blood glucose response curve elicited by a 50g portion of available carbohydrate of a test food expressed as a percentage of the response after a 50g portion of glucose (reference food) taken by the same subject (Wolever et al., 1991b). The GI is a property of the food, and is often times confused with the glycemic response, which varies from subject to subject (Wolever, 2006). The reference food is therefore used to correct for between-subject variation, as the GI adjusts the glycemic responses of the test food to each subject’s individual response to the reference. This allows for the comparison of different foods that are measured across different subject
groups (Wolever et al., 1990). A standard protocol for determining the GI of foods exists (FAO, 1998), which is described in detail in the methods chapter of this thesis. Using this method carbohydrate containing foods can be classified as either low (≤55), intermediate (56-69) or high (≥70) GI, based on the glucose reference scale (GI=100) (Atkinson et al., 2008).

As mentioned, studies have shown that the rates of digestion of foods in-vitro are related to their glycemic responses in vivo, suggesting that differences in GI are due to differences in the rates of digestion and absorption of carbohydrate from different foods (Englyst et al., 1999; Englyst et al., 2003; Englyst et al. 1996b). This is evident in a study investigating the determinants of GI on 23 manufactured cereal products. Results indicated that in-vitro measures of RAG and SAG (i.e. the rate of carbohydrate digestion) were closely related to the GI and described 68% of the variance in GI between foods (Englyst et al., 2003). The importance of type of carbohydrate was also noted in a study conducted by Wolever & Bolognesi (1996), where subjects were fed varying amounts and sources of carbohydrates (total carbohydrate minus dietary fibre), to determine whether the source and amount of carbohydrate affected postprandial glucose and insulin responses. They found that 46-64% of the variability between glucose and insulin responses could be explained by the source of carbohydrate similar to the 47-57% of the variability explained by the amount of carbohydrate. This study concluded that alone both the amount and source of carbohydrate are poor predictors of postprandial glucose and insulin. However, these results validate the importance of considering and thus measuring the source (GI) of carbohydrate foods along with the amount consumed.
From its discovery by Jenkins et al. (1981), until around the mid 1990s the only clinical application of the GI was considered to be in the treatment of diabetes. Since then interest in the GI and its utility has increased exponentially due to its newfound relevancy to normal populations and different aspects of human health (Wolever, 2006). Although the GI has gained popularity over the years, conflicting views remain around the reliability and application of this measure in clinical and public health settings. Many GI skeptics continue to question its usefulness for a number of different reasons (Flint et al., 2004; Venn & Green, 2007; Williams et al., 2008; Vega-López et al., 2007; Coulston et al., 1984), even though most if not all of these issues have been addressed elsewhere (Wolever et al., 1991b; Wolever et al., 2008; Chew et al., 1988).

In addition to the GI, other in-vivo methods such as the ileostomy model and breath hydrogen technique have been used to investigate carbohydrate digestibility. More specifically they have the ability to quantify the amount of malabsorbed carbohydrate, or RS in a food.

2.2.3 Ileostomy Model

An ileostomy is a surgically created opening where the ileum is brought to the abdomen, diverting effluent into a pouch instead of its normal route through to the colon. It is often used in treatment for disease or trauma to the colon including inflammatory bowel disease and familial adenomatous polyposis, where the colon is either removed, or requires time to heal (The Canadian Association for Enterostomal Therapy, 2009). Thus, these patients have been used to study small intestine digestion and absorption, as undigested nutrients are collected and then measured in the newly created abdominal
pouch. Briefly, the experimental design for most ileostomy studies include a period where subjects with an ileostomy are placed on a control diet that is low or free of the nutrient of interest (i.e. starch), followed by a period where the nutrient is included in the diet. Diet information and ileal effluent is then analyzed to determine the presumed absorption or malabsorption of the nutrient (Wolever, 2006).

The digestibility of starchy foods, including potato products measured in-vitro has been reflected in in-vivo ileostomy studies. The amounts of starch recovered in the ileostomy studies however are typically higher than the RS values obtained in-vitro. This is likely due to variation between the individual ileostomy subjects, as the in-vitro methods depend only on the property of the food and not on extrinsic factors such as individual chewing habits or gut transit time (Englyst & Cummings, 1985; Englyst et al., 1992b).

The amounts of malabsorbed carbohydrate measured in ileostomy effluent have also been compared to the glycemic response. A study conducted by Wolever et al. (1986) found that the amount of malabsorbed carbohydrate recovered in the effluent after each test food was not related to the foods’ area under the blood glucose curve (AUC). While, another study by the same group (Jenkins et al., 1987) looked at the digestibility and glycemic response of 20 starchy foods in one or more ileostomate subjects and found that the glycemic response was negatively correlated ($r=-0.611, p<0.05$) with the percent carbohydrate malabsorbed after each of the test foods. However, Wolever (2006) showed that if you theoretically reduce carbohydrate intake by an amount equal to the proportion of carbohydrate malabsorbed it only explains a small proportion of the reduction in glycemic response. This is supported by Fernandes et al. (2005), as carbohydrate malabsorption in cooked and cooled potatoes (measured by the ileostomy model in
Englyst et al. (1987)) accounted for only about 15% of the 35% reduction in glycemic response upon cooling a boiled red potato. This suggests that differences in glycemic responses between foods could be better explained by differences in the rate of carbohydrate digestion rather than the amount.

To summarize ileostomy models are able to quantify carbohydrate malabsorption and provide a more physiological representation of starch digestibility than in-vitro methods. However, this measurement of malabsorption in-vivo, cannot account for the entire reduction in glycemic response and unlike in-vitro methods cannot identify different rates of starch digestion. In recent years although ileostomy studies have generally been considered a reliable model for small intestine absorption other in-vivo methods such as breath hydrogen have been used more often. This is likely due to difficulties in recruiting subjects, inconveniences of consuming restricted diets and the collection of effluent (Wolever, 2006).

2.2.4 The Breath Hydrogen Method

Hydrogen gas (H₂) is produced in the colon by anaerobic bacteria, as a byproduct of fermented un-digested carbohydrate (i.e. RS). H₂ diffuses through the colonic epithelium into circulation, where it is carried to the lungs and excreted through breath (Read et al., 1985). Analyzing expired air for hydrogen concentration is a non-invasive method of quantifying carbohydrate malabsorption, which was validated by Bond et al. (1972) and has been used ever since (Anderson et al., 1981; Flourie et al., 1988; Robb et al., 1991; Rumessen et al., 1990; Levitt et al., 1987; Rumessen et al., 1994; Owira et al., 2005).
Briefly, breath hydrogen is typically measured over an 8-12 hr period after the consumption of a carbohydrate test meal at 30 min or hourly intervals. One of the test days involves subjects taking a known dose of an un-absorbable standard carbohydrate, most often lactulose or inulin, followed by a series of other test days including known amounts of each test carbohydrate. Breath hydrogen production after each of the test meals is typically measured by calculating the AUC or sum of the individual concentrations. Lactulose or inulin is used to determine the $H_2$ produced per gram of unabsorbable carbohydrate taken by each subject, this value is then divided into the amount of $H_2$ produced from each of the other test meals to give the grams of carbohydrate malabsorbed (Wolever, 2006). Therefore, this technique is only semi quantitative in nature.

The $H_2$ breath method has been criticized, but has generally been considered a valid technique for assessing carbohydrate malabsorption (Rumessen, 1992). Common criticisms include large within and between subject variation (Flourie et al., 1988) and previous antibiotic use (Gilat et al., 1978). Examples of within subject variation include, content, amount and timing of meals before the test day, or retention of feces and gas in the colon (Robb et al., 1991). A common example of between subject variation would be, differences in colonic flora composition (Gilat et al., 1978). There is also the possibility that the technique may not be sensitive enough to detect small amounts of malabsorbed carbohydrate (i.e. less than 10g) (Grysma et al., 2008) and the fact that certain malabsorbed carbohydrate when fermented by colonic bacteria do not produce $H_2$ (Sanz et al., 2005; Livesey et al., 1993; Olesen et al., 1994; Wolever & Robb, 1992).
A study by Flourie et al. (1988) evaluated the accuracy of the H\textsubscript{2} breath test with lactulose as the indicator carbohydrate for quantifying the malabsorption of raw wheat starch (thought to be resistant to small intestine digestion). Subjects were rectally infused with different amounts of starch that were compared to respective calculated amounts which were determined through measurement of breath H\textsubscript{2}. Calculated results were significantly correlated with the actual quantities of starch infused. This suggests that the lactulose breath test is a valid method for comparing starch absorption in different foods. However, Flourie et al. (1988) also concluded that although the mean calculated values did not differ significantly from the infused amounts, there was significant intra-individual variation and therefore suggested this measurement is not appropriate for evaluating starch malabsorption in a given subject.

Another study conducted by Wolever et al. (1986) validated the breath H\textsubscript{2} method and showed it to be remarkably similar to the direct measurement of the amount of undigested carbohydrate in the small intestine in subjects with an ileostomy after three different test foods. From this study the estimated mean percentage of available carbohydrate malabsorbed from white bread, whole wheat bread and red lentils measured by the breath H\textsubscript{2} method was 10\%, 8\% and 22\%, respectively, compared to 11\%, 8\% and 18\%, respectively using the ileostomy model. However, for this study, as previously discussed, the amounts of malabsorbed carbohydrate after each of the different foods as determined by the ileostomy model were not related to their glycemic response. It is therefore assumed that the un-absorbed carbohydrate determined through breath H\textsubscript{2} for these foods was also not related to the glycemic response. Additionally, as discussed by Wolever (2006) concerning the ileostomy model, the amount of carbohydrate malabsorbed
measured by breath hydrogen may account for some of the reduction in glycemic response, but cannot explain all of the reduction, as indicated in Wolf et al. (2003).

In summary, the breath H\textsubscript{2} technique is a non-invasive way of detecting carbohydrate malabsorption and similar to the ileostomy model is generally considered a valid method. Both methods, however are unable to evaluate the different rates of starch digestibility, which may be more appropriately measured in-vivo through stable isotopes or the euglycaemic hyperinsulinaemic clamp (not discussed here) (Wolever, 2006). Perhaps the combination of both in-vivo and in-vitro methods is most appropriate to investigating differences in starch digestibility between foods.

2.3 Health Implications of RDS, SDS and RS

Following a review of the methods commonly used in the classification of carbohydrate digestion, this section will briefly re-visit the characterization of each starch fraction and discuss their health implications, in an attempt to justify the importance of these methods in identifying carbohydrate foods that are rapidly, slowly or resistant to digestion.

2.3.1 RDS

RDS or RAG is rapidly digested and absorbed in the duodenum and proximal regions of the small intestine. This results in rapid elevation and high peak of blood glucose, usually leading to a fast decline, partially below baseline levels (Lehmann & Robin, 2007; Zhang & Hamaker, 2009). Foods thought to be high in RDS, are typically classified as high GI. RDS and high GI have been associated with negative health outcomes. For example, high GI diets and RDS contribute to potential risk factors for metabolic syndrome such as,
greater and more rapid changes in blood glucose, insulin and non esterfied fatty acids (NEFA) concentrations (Ells et al., 2005; McKeown et al., 2004; Seal et al., 2003). Ells et al. (2005) suggested an exchange of SDS for RDS in the diet to reduce these potentiating risk factors. Brownlee et al. (2005) noted that a high concentration of cellular glucose uptake resulting from high chronic consumption of RDS can lead to production of free radicals in the mitochondria resulting in oxidative stress. An increase consumption of high GI foods however has been recommended practice amongst certain types of populations (i.e. athletes) (Burke et al., 1996; Wee et al., 2005) although is not always recommended (Stevenson et al., 2009).

2.3.2 SDS

Unlike RDS, SDS or SAG can be characterized by a slow increase in postprandial blood glucose levels, as well as its ability to sustain blood glucose levels over this period (Lehmann & Robin, 2007). Foods with a greater proportion of SDS than RDS, are usually measured as intermediate-low GI (Englyst et al., 2003; Lehmann & Robin, 2007). Epidemiological studies have suggested that low-GI diets may play a role in reducing the risk of CVD, diabetes, and certain cancers (Wong & Jenkins, 2007). A meta-analysis of randomized control trials was conducted by Brand-Miller et al. (2003) to investigate the effects of a low GI diet on overall glycemic control (indicated by glycated hemoglobin ($\text{HbA}_{1c}$) or fructosamine) compared to a high GI diet in subjects with type 1 and type 2 diabetes. This analysis indicated that low GI diets significantly reduced $\text{HbA}_{1c}$ and fructosamine by an average of 7.4% more than on the high GI diet (95% CI 8.8-6.0) (Brand-Miller et al., 2003). However, a 12-month study, looking at the effects of altering the GI or carbohydrate intake on various bio-measures associated with the management
of type 2 diabetes found that a low GI diet had no effect on HbA1c. C-reactive protein (CRP) however, was 30% less in subjects on the low GI diet than on the high and a sustained reduction in postprandial glucose was also noted (Wolever et al., 2008). There is also evidence suggesting that slowing and prolonging the rate of absorption (i.e. a meal higher in SDS) can prolong postprandial suppression of free fatty acids (FFA) and improve insulin sensitivity. Which reduces the glycemic impact of a subsequent meal, commonly referred to as the “second meal effect” (SME) (Jenkins et al., 1982; Axelsen et al., 1999; Wolever et al., 1995).

2.3.3 RS

Finally, RS is not digested in the upper gastrointestinal tract, and passes through to the colon where it is fermented by colonic bacteria. Thus, this starch fraction does not influence blood glucose levels, directly (Zhang & Hamaker, 2009; Sajilata et al., 2006). Foods containg RS can moderate or slow the rate of digestion of more absorbable starches (Sajilata et al., 2006). The byproducts of colonic fermentation of non-digestible carbohydrates are the short chain fatty acids (SCFA), acetate, propionate and butyrate. Starch fermentation primarily yield acetate and butyrate. Butyrate specifically, has been positively associated with colonic health, as it is the primary fuel for colonocytes and assists in the regulation of cell proliferation and differentiation (Wong & Jenkins, 2007). Both acetate and propionate have been proposed to have opposing effects on lipid metabolism. Acetate for example, may have the ability to lower serum FFA, (Wolever et al., 1989; Wolever et al., 1991a; Ferchaud-Roucher et al., 2005) but raise cholesterol and triglycerides as well (Wolever et al., 1989; Wolever et al., 1991a), although other studies have reported no effect on cholesterol and a reduction in triglycerides (Vogt et al., 2006).
There is evidence to support the idea that propionate inhibits the utilization of acetate for cholesterol synthesis (Wolever et al., 1991a). Studies using oral rather than rectal infusions have been inconsistent, as one study showed no effect on serum cholesterol and reported elevated triglycerides (Venter et al., 1990), while another study reported a reduction in serum cholesterol (Vogt et al., 2006). Animal studies have also suggested that propionate inhibits cholesterol synthesis (Wong & Jenkins, 2007). Finally, RS can be considered a prebiotic as it has been linked to the promotion of beneficial bacteria such as *Bifido bacterium* (Sajilata et al., 2006).

In summary due to the number of health outcomes associated with different rates of carbohydrate absorption, classification systems are important in providing consumers with the opportunity to limit or consume more of certain types of foods based on their glycemic impact. It is sometimes difficult however to classify a type of food; especially starchy foods with a specific GI value. As mentioned there are a number of factors that can contribute to differences in the rate and extent at which carbohydrates are absorbed by the small intestine. These factors should be taken into account when measuring and interpreting the GI values of foods. The following section of this literature review will discuss the potential factors that affect the digestibility of potatoes and the current literature which report GI values to formulate a basis for its purported high GI.

### 2.4 Potato Digestibility and the Glycemic Index

As previously mentioned the main carbohydrate in potatoes is starch. A raw potato contains mostly RS, which is resistant to digestion and absorption by the small intestine. For example, a study that used the Englyst et al. (1992a) method to measure RDS, SDS
and RS fractions in potato samples processed differently reported a raw Marfona potato to contain 70% RS, 7% SDS and only 2% RDS (% of total dry matter) (Kingman & Englyst, 1994). Other in-vitro studies looking at different varieties have found similar results in terms of high RS content, however the proportion of starch fractions differ slightly depending on variety (H. Englyst & Cummings, 1987; Liu et al., 2007; Mishra et al., 2008), which could be due to a number of factors. These factors, along with how they could potentially affect the digestibility of potatoes will be discussed in the next section. Based on these in-vitro studies it is presumed that raw potatoes could only slightly if at all influence blood glucose as they contain insignificant amounts of available carbohydrate. Since potatoes are unlikely to be consumed raw, there are no studies to our knowledge that have tested the glycemic response of raw potato starch. It can only be presumed from the in-vitro data, that raw potatoes would have a very low or an un-measurable GI, due to their negligible RDS and SDS contents.

In order to consume potatoes, they are usually boiled, baked, roasted, steamed or fried, causing starch granules to gelatinize (process explained in section 2.1.2) resulting in the transition from a RS to a more digestible fraction (i.e. RDS). Using the same study as above, upon boiling a Marfona potato the amount of RDS climbs to 71% of dry matter, while SDS and RS both drop to 1% (Kingman & Englyst, 1994). Other in-vitro studies noted similar proportions of the starch fractions after cooking (Mishra et al., 2008; Englyst et al., 1992a; Leeman et al., 2005). When potatoes are cooled, gelatinized starch retrogrades (process explained in section 2.1.2) resulting in a large proportion of RDS converting to SDS or back to RS. When the boiled Marfona potato was cooled, for example RDS fell to 66% of dry matter, while SDS and RS climbed to 9% and 5%
respectively (Kingman & Englyst, 1994), this has also been indicated by other studies upon cooling previously cooked potatoes (Englyst et al., 1992a; Leeman et al., 2005; Mishra et al., 2008).

The changes in terms of starch structure that occur after cooking and cooling potatoes are not only indicated by in-vitro measurements of starch digestibility but also through glycemic responses and measurement of the GI. Referring to some of the most recent papers reporting GI values for potatoes (Fernandes et al., 2005; Henry et al., 2005; Leeman et al., 2008; Soh & Brand-Miller, 1999; Leeman et al., 2005) most reported the GI of fresh cooked potatoes to be greater than 70 (high GI), with a range of 72-118 on the glucose scale (GI=100). All studies utilized the standard protocol for measuring the GI (FAO, 1998), but were not all cooked in the same way and varieties differed across studies. The range in GIs does not appear to be explained by different cooking methods, as Soh & Brand-Miller (1999) reported no significant differences between GI values for baked, boiled and mashed, boiled and microwaved Pontiac potatoes (93±11, 91±9, 88.9±9, 79±9, respectively). Fernandes et al. (2005) reported similar findings, as a baked Russet, roasted California white, baked PEI white, and a boiled red, also did not differ significantly in terms of their GI (76.5±8.7, 72.3±8.2, 72.8±4.5, 89.4±7.2, respectively). The Fernandes et al. (2005) study also suggests that the variation in GI values (72-118) between studies may not be explained by difference in varieties. This theory is also supported in Soh & Brand-Miller (1999), as there were no significant differences between the GI of boiled Desiree, Sebago or Pontiac potatoes (101±15, 87±7, 88±9, respectively). However, comparing the same variety (Desire) and cooking method (boiling) across two different studies, indicated quite different GIs of 77±17 (Henry et al.,
2005) and 101±15 (Soh & Brand-Miller, 1999). This variation could be due to experimental error, or environmental factors such as time or location of harvest (discussed further in following section), as the potatoes tested in Henry et al. (2005) were purchased in the United Kingdom while the potatoes from the Soh & Brand-Miller. (1999) study were purchased in Sydney, Australia from retail supermarkets. In terms of cooling, only the Fernandes et al. (2005) and Leeman et al. (2005) studies subsequently cooled previously cooked potatoes. Firstly, the GI of a boiled red potato (hot) was 89.4±7.2 which dropped to 56.2±5.3 when cooled in a refrigerator for 12-24 hrs (Fernandes et al., 2005). The Leeman et al. (2005) study, reported similar results, as the GI for a boiled Sava potato was 118, and upon cooling for 24 hrs at 8°C the GI fell to 88, this reduction in GI however was not significant. Although the GI for both varieties was reduced upon cooling the red potato from the Fernandes et al. (2005) study reached significance. This difference between varieties in terms of the magnitude at which cooling effected the GI, could be due to different physicochemical properties found in each variety (discussed below).

Reheating cold potatoes has been shown in-vitro to restore some of their digestibility, by the re-gelatinization of retrograded starch (Englyst & Cummings, 1987; Kingman & Englyst, 1994). There have been very few studies however, measuring the effects on the GI after reheating Fernandes et al. (2005), measured the glycemic response but not the GI of day cooked and pre-cooked (reheated); Russet Burbank, baked (conventional oven), Russet Burbank, baked (microwave) and California white, boiled potatoes. They found that there was no difference in terms of mean blood glucose AUC between the day cooked and pre-cooked for both the Russet Burbank, baked (microwaved) and California
white, boiled potatoes. However, mean AUC after precooked Russet baked in a conventional oven was 30% less than day-cooked potatoes, a difference which was statistically significant (Fernandes et al., 2005).

There has also been some investigation around the effects of temperature cycling and the duration and extent of cold treatments on starch digestibility and the GI (Englyst & Cummings, 1987; García-Alonso & Goñi, 2000; Kingman & Englyst, 1994; Fernandes et al., 2005; Leeman et al., 2008). For example, French fries which are pre-cooked, frozen and then reheated, typically by frying or baking tend to have lower GIs than freshly cooked potatoes. Fernandes et al. (2005), reported the GI of baked French fries to have an intermediate GI of 63.6±5.5, which was significantly lower than the boiled red potato (89.4±7.2). Leeman et al. (2008), reported a low GI of 54 for baked French fries tested in their study, which was significantly lower than Asterix boiled potatoes (78). These relatively low GIs may be explained by the pre-cooking, freezing and then reheating process which has showed to create more SDS and RS fractions in-vitro (García-Alonso & Goñi, 2000). Another theory is the formation of amylase-lipid complexes formed during frying and/or reheating which are more slowly digested or resistant to α-amylase (García-Alonso & Goñi, 2000). It is obvious that different processing methods can affect the digestibility of a potato, which ultimately affects its GI. Certain physicochemical properties of the starch may influence how different potato varieties are affected by heating and cooling treatments. These properties will be discussed in the following section, along with different environmental factors, that could affect them.
2.4.1 Physicochemical Factors Influencing Starch Digestibility and the GI

The following briefly reviews some of the physicochemical properties and environmental factors that have been indicated as factors in influencing potato starch digestibility.

2.4.1.1 Amylose: amylopectin ratio

The ratio of amylose to amylopectin within potato starch is considered the most important factor affecting the rate and extent of hydrolysis (Zhang et al., 2008). As previously discussed starch granules typically contain amylose and amylopectin in a ratio of 1:3 (Camire et al., 2009). This ratio can explain why cooked potatoes typically have high GIs, as amylopectin due to its branched structure, is the polysaccharide that is readily gelatinized (Camire et al., 2009). Potato starch containg higher degrees of amylose, would likely result in less gelatinized starch upon heating resulting in more SDS or RS and a lower GI. A high degree of amylose may also be advantageous upon cooling. As it is linear amylose that is, better able to re-associate to form a crystalline (retrograded) structure that is more resistant to digestion (Camire et al., 2009).

2.4.1.2 Amylopectin Branching

Identifying the fine structure of amylopectin, namely the chain length distribution and its branching pattern is important to understanding starch digestibility as well (Zhang et al., 2008). Amylopectin containg a greater number of branches has a lower gelatinization temperature, and amylopectin with a greater number of long chains requires a higher gelatinization temperature (Zhang et al., 2008). This study also indicated starch samples with amylopectin that had either a high proportion of short chains or a high proportion of
long chains contained a higher content of SDS. Therefore the Zhang et al. (2008) study indicated at least two types of amylopectin structures that are characteristic of SDS fractions. Longer chain length and high amount of longer chain fractions in the starch molecule has a potential role in starch retrogradation as well (Englyst, Kingman, & Cummings, 1992a). A study by Liu et al. (2007) reported a Karnico potato to contain higher amylose content and a greater proportion of long chains, which resulted in the highest resistant starch content of any of the other varieties tested in both its native and gelatinized form.

2.4.1.3 Phosphorus Content

As mentioned, potatoes have quite marketable phosphorus contents, and besides its nutritional implications, phosphorus indirectly affects starch digestion and absorption as it plays an important role in gelatinization and retrogradation processes. In potato starch, the phosphorus is mainly present as phosphate monoesters, which are covalently bound to amylopectin and thus it has been reported as being negatively correlated with linear amylose (Karim et al., 2007). Phosphorus has also been noted to facilitate the swelling of amylopectin during the gelatinization or heating process (Karim et al., 2007). Since phosphorus is associated with amylopectin, which is readily gelatinized compared to amylose, high phosphorus content can be linked with a more rapid rate of starch digestion and absorption.

2.4.1.4 Year of Harvest and Environmental Influences

Year to year variation within the same variety with regards to the physicochemical properties of potato starch have been reported (Svegmark et al., 2002). Variation in
growing conditions (i.e. weather, growing location) has been shown to influence
gelatinization behavior of the starch, granular size, amylose and phosphorus content, both
within and between varieties (Haase & Plate, 1996; Cottrell et al., 1995).

2.4.1.5 Maturity

There is evidence that small or “new” potatoes have slower rates of digestion compared
to larger or more mature ones resulting in a lower GI value (Soh & Brand-Miller, 1999).
It has been suggested that the reduction could be due to the fact that less mature potatoes
have a lesser degree of amylopectin branching, which leads to a higher resistance to
gelatinization, resulting in a slower rate of starch hydrolysis indicated by a low GI (Soh
& Brand-Miller, 1999). An in-vitro study however, did not report significant differences
between early and late potatoes and attributed the observed differences in the Soh &
Brand-Miller (1999) study to be due to some other food factor (Leeman et al., 2005). A
study by Henry et al. (2005) found similar results as Soh & Brand-Miller (1999), in that
earlier crop varieties generally had lower (intermediate GI=56-69) GIs than the main or
late crop varieties (high GI≥70).
Chapter 3

Rationale, Objectives, Hypotheses

3.1 Rationale

Based on the literature, both in-vitro and in-vivo studies have confirmed that freshly cooked potato starch generally, is rapidly digested and absorbed in the small intestine, causing a more rapid rise in postprandial glucose. This has lead to the classification of potatoes as a high GI food. Due to the association of high GI diets with negative health outcomes such as diabetes and cardiovascular disease, potatoes have the potential of being limited or even excluded from the diet, and from recent potato consumption data this may already be the case (AAFC, 2007).

What are not always considered in classifying starch based on its digestibility are factors such as; variety, preparation methods, physicochemical properties of the starch (i.e. ratio of amylopectin to amylose), harvest date/year and growing conditions. Based on review of the literature there is evidence to suggest that these factors can influence each other thereby influencing the digestibility of the potato itself. This suggests that not all potatoes can be labeled with one high GI value. If the goal, ultimately is to increase overall intake of foods high in SDS and RS content due to their associated health benefits, than it is important to obtain a better understanding of these factors and their effects on the glycemic response.

A network of scientists from across the country, including plant breeders and starch chemists (Bio Potato Network (BPN)) are in collaboration in order to identify specific
genotypes/germplasm with properties such as high amylose content and special branch structures of amylopectin. Their overall goal is to ultimately develop or breed potato starch that is slowly or less absorbed, resulting in new potato varieties with low GIs. We examined a total of four such varieties (not available commercially), which differed with respect to their amylose and fibre contents, to investigate how varying these and other factors may affect the GI of potatoes. We previously showed that the effects of certain preparation methods on the GI may depend on potato variety (Fernandes et al., 2005). Thus, a pilot study (study 1) was designed to determine whether we could use one preparation method for all varieties, or whether it was important to test various methods for all varieties received. This led to the development of our first objective. Further objectives were established based on the results from study 1. A total of three studies were developed in accordance with the following objectives and hypothesis.

### 3.2 Objectives

1. To determine if the effects of cooking, cooling and reheating on the GI of potatoes differs depending on the variety.

2. To confirm the effects of cooking and cooling on the GI in a subsequent harvest year on varieties that were affected differently by these treatments in study 1.

3. To investigate the rate and extent of starch absorption after cooked and cooled potato meals to determine whether the reduction in GI upon cooling can be explained by the formation of RS or by the creation of SDS.
3.3 Hypotheses

1. The effects of cooking, cooling and reheating on the GI of potatoes will differ depending on variety.

2. The effects of cooking and cooling on the GI of potatoes noted in study 1 will be confirmed in a subsequent harvest year.

3. A small amount of RS will form when a cooked potato is cooled, however the creation of SDS will explain the reduction in GI
Chapter 4

Methods

4.1 Study 1

4.1.1 Overview

During study 1, GI testing was conducted on four different potato varieties, harvested in Fall 2008 to determine whether cooking, cooling and reheating processes affected the varieties differently. This study took place from February-April 2009.

4.1.2 Subjects

Twenty healthy subjects participated in this study. Subjects were divided randomly into two equal groups. Group A consisted of five females and five males with a mean age of 20.1± 0.7 years, mean height of 171.9 ± 2.0 cm, mean weight of 73.2 ± 4.5 kg, and mean BMI of 24.6± 1.1 kg/m². Group B consisted of six females and four males, with a mean age of 20.5± 0.5 years, mean height of 170.9± 2.6 cm, mean weight of 68.7± 3.3kg, and mean BMI of 23.7± 0.7 kg/m². One male subject from group B could no longer participate after one test visit, thus a male subject from group A completed the remainder of the potato tests assigned to group B in addition to his tests for group A.

Subjects were excluded if they reported having diabetes mellitus or glucose intolerance as well as any metabolic or gastrointestinal disease that interfered with nutrient absorption. Subjects were free of medications that interfered with nutrient absorption, metabolism, excretion or gastric motility and had not taken corticosteroids within three months or
antibiotics within three weeks of starting the study. All subjects provided written
informed consent before participation. The Research Ethics Board at the University of
Toronto granted ethics approval.

4.1.3 Test Meals

Four potato varieties, which differed with respect to their starch composition and fibre
content, were tested. These varieties were not available commercially during the study
period and will therefore be identified as varieties 1, 2, 3 and 4. Varieties 1 and 2 were
grown in Vauxhall, Alberta while varieties 3 and 4 were grown in Fredericton, New
Brunswick. All varieties were harvested during the 2008 season. Potatoes were stored at
Guelph University, at 11ºC, until required for the study. All varieties were sprout
inhibited, prior to their shipment to Guelph to prevent spoilage. Once at the lab potatoes
were stored in a dark cupboard at ambient temperature.

In order to complete the study in a timely manner, subjects in group A tested varieties 1
and 2 and group B tested varieties 3 and 4. Each variety was boiled and served to subjects
either fresh boiled, cold or reheated. Thus each subject in each group consumed six
potato test meals. In terms of preparation, on the morning of each test day potatoes were
cut into 2.5-3cm cubes and then weighed into predetermined portions (described below).
Individual portions (potato meals) were boiled in salted water with skin intact for 15
minutes, and then drained. Boiled potato meals were either served to subjects
immediately (fresh boiled) or they were cooled in the refrigerator at 4ºC and either served
cold or reheated to subjects 24-48 hrs later. Reheated potato meals, were warmed in the
microwave for 2-3 min and then served.
In addition to the potato meals, all subjects consumed a test meal of baked potato chips. This served as a positive control, to verify that the GI for this food was the same in both subject groups, to provide validity for the between group comparisons of GI values of the different potato varieties. Each subject also consumed a test meal of white bread, on three separate occasions, which served as a reference food for GI determination. White bread was baked from weighed ingredients in a bread maker as previously described (Wolever et al., 2003).

All test meals contained 50g available carbohydrate (defined as total carbohydrate minus dietary fibre). For each variety, total carbohydrate and fibre content was determined by proximate analysis. Proximate analysis was performed by Agri-Food Laboratories in Guelph, Ontario and analysis for total dietary fibre was performed by Maxxam Analytics in Mississauga, Ontario. Results from this analysis were given in 100g fresh weight units and were then used to determine the weight of each variety to feed to ensure each test meal contained 50g of available carbohydrate. Test meal composition of varieties 1, 2, 3 and 4 are presented in Table 1. The composition of the baked potato chip test meal was determined by its nutrition facts table, and is presented in Table 1. The composition of the reference white bread was predetermined using proximate analysis and has been frequently re-analyzed (Table 1).

4.1.4 Protocol

The study was conducted at Glycemic Index Laboratories in Toronto, Ontario. Each test lasted for 2 hours (120 min), and subjects were required to leave at least 48 hrs between visits. Subjects arrived at the lab between 8-9:30am on the morning of the test after
Table 1. Test meal composition for Study 1

<table>
<thead>
<tr>
<th>Test Meals</th>
<th>Weight (g)</th>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Total CHO (g)</th>
<th>Fibre (g)</th>
<th>Available CHO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>345</td>
<td>252</td>
<td>0</td>
<td>7.2</td>
<td>55.8</td>
<td>5.8</td>
<td>50.0</td>
</tr>
<tr>
<td>Variety 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>360</td>
<td>256</td>
<td>0</td>
<td>6.5</td>
<td>57.2</td>
<td>7.2</td>
<td>50.0</td>
</tr>
<tr>
<td>Variety 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>321</td>
<td>244</td>
<td>0</td>
<td>6.1</td>
<td>55.6</td>
<td>5.4</td>
<td>50.0</td>
</tr>
<tr>
<td>Variety 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>394</td>
<td>256</td>
<td>0</td>
<td>7.1</td>
<td>56.3</td>
<td>6.3</td>
<td>50.0</td>
</tr>
<tr>
<td>Baked Lays potato chips</td>
<td>66</td>
<td>264</td>
<td>3.3</td>
<td>5.3</td>
<td>54.1</td>
<td>4.0</td>
<td>50.0</td>
</tr>
<tr>
<td>White bread&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104</td>
<td>245</td>
<td>1.7</td>
<td>7.5</td>
<td>52.8</td>
<td>2.8</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Abbreviations: CHO, carbohydrate

<sup>a</sup> Proximate analysis performed by Agri-Food Laboratories, Guelph (Moisture: AOAC 930.15 (not shown), Protein: AOAC 990.03, Ash: AOAC 942.05 (not shown), Fat: AOAC 920.39, Carbohydrate: by difference); Analysis for Total Dietary Fibre performed by Maxxam Analytics, Mississauga (Total Dietary Fibre: AOAC 993.43, 985.29).

<sup>b</sup> Typical portion size, actual weight varied slightly due to moisture variations.
fasting for 10-12 hours. Each subject came to the lab on 10 separate mornings (6 potato tests, 1 baked potato chip test and 3 white bread tests). Upon arrival, subjects were weighed and two fasting blood samples (-5min and 0min) were collected by finger-prick using a Microlet lancing device (Bayer Diagnostics, Tarrytown, NY, USA). Subjects then consumed one of the test meals described above plus a drink of 250ml of water, tea, or coffee with 30ml of 2% milk if desired (type and volume of beverage chosen by each subject remained the same for the entire series of test foods). Test meals were consumed within 15 minutes and within each group, each subject consumed all test meals in a randomized order. Additional finger-prick blood samples (2-3 drops) were taken at 15, 30, 45, 60, 90, and 120 minutes after the start of the meal. Blood was collected into fluoro-oxalate tubes, stored at -20° C, before analysis for glucose.

4.1.5 Analytical Methods

Finger-stick whole blood glucose for each time point was analyzed using a Model 2300 STAT analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA).

Dr. Q. Lui’s lab at Agriculture, Agri Food Canada (AAFC) provided physiochemical data for all four varieties, to correlate with the GIs of the potatoes. Starch was isolated from the varieties following the methods described in Liu et al. (2003). Amylose of potato dry matter was determined by iodine colorimetry according to Williams et al. (1970) (Table 2). RDS, SDS and RS were also obtained from AAFC. Cooked starch samples from each variety were treated with pancreatic α-amylase and amyloglucosidase and incubated for 20 and 120 minutes. Starch digestibility in vitro was measured based on the Englyst et al. (1992a) method. Starch classifications were based on the rate of hydrolysis with; RDS,
Table 2. Amylose, RDS, SDS and RS content of potato starch*

<table>
<thead>
<tr>
<th>Potato</th>
<th>Amylose in dry matter (%)</th>
<th>RDS in potato starch (%)(^a)</th>
<th>SDS in potato starch (%)(^a)</th>
<th>RS in potato starch (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety 1</td>
<td>27.1 ±0.2</td>
<td>73.8± 0.2</td>
<td>3.7± 2.1</td>
<td>22.5</td>
</tr>
<tr>
<td>Variety 2</td>
<td>24.3±0.2</td>
<td>71.1± 2.5</td>
<td>4.6± 1.9</td>
<td>24.3</td>
</tr>
<tr>
<td>Variety 3</td>
<td>22.7±0.2</td>
<td>68.7± 1.9</td>
<td>7.4± 1.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Variety 4</td>
<td>22.8±0.1</td>
<td>68.5± 1.8</td>
<td>5.8± 0.8</td>
<td>25.7</td>
</tr>
</tbody>
</table>

Abbreviations: RDS, rapidly digestible starch, SDS, slowly digestible starch, RS, resistant starch.

*Values denote means ± SEM. Values for amylose obtained from the mean value of two raw potato samples for each variety. RDS, SDS obtained from the mean values of two cooked starch samples for each variety.

\(^a\) RDS, SDS based on rate of starch hydrolysis with RDS classified as starch digested within 20 minutes and SDS classified as starch digested between 20-120 minutes (Englyst et al., 1992a)

\(^b\) RS determined by: (100- (% RDS + % SDS)) and is classified as undigested starch after 120 minutes (Englyst et al., 1992a)
digested within 20 minutes, SDS, digested between 20-120 minutes and RS, undigested after 120 minutes (Table 2). TS was not provided however, in order to determine RS it was suggested to use 100, in place of the TS value.

4.1.6 Calculation of Glycemic Index

Incremental areas under the blood glucose curve (AUC), excluding area below fasting, were calculated using the trapezoid rule (FAO, 1998). The AUC for each potato test meal in each subject was expressed as a percentage of the mean AUC for the white bread test meals taken by the same subject resulting in that subject’s individual GI. The GI for each potato meal was then taken as the average of all ten individual subject values.

Both glucose and white bread have been used as the reference food for GI testing. To reduce confusion it has been recommended that cited GI values be expressed on the glucose scale (Wolever et al., 2003). Thus the GI values from this study were converted to glucose-based GI values (i.e. GI of glucose= 100) by multiplying the bread based GI values by 0.71, as white bread elicits a glycemic response 29% less than that of oral glucose (Wolever et al., 2003).

4.1.7 Statistical Analysis

Results are expressed as means ± standard error of the mean (SEM). To determine if the GI of baked potato chips differed between group A and group B a two-tailed un-paired t-test was conducted.

Blood glucose profiles for each variety (1,2,3, and 4) and treatment (fresh boiled, cold, and reheated) were subjected to analysis of variance (ANOVA) to identify a possible
time× treatment × variety interaction. If this three way interaction was significant, data from each time point was subjected to ANOVA examining for the effects of treatment and variety. AUC and GI values were also subjected to ANOVA examining for the main effects of potato variety and treatment and the variety×treatment interaction. If the variety×treatment interaction was significant, data from each variety was subjected to ANOVA examining for the effects of treatment. After demonstration of significant heterogeneity, the significance of differences between individual mean blood glucose concentrations at each time point, AUC and GI values were determined using the Bonferroni method to adjust for multiple comparisons. A separate analysis was also conducted to compare the GIs of each potato test meal to that of white bread (GI white bread= 71). This analysis involved only one factor (food) and thus a one-way ANOVA was conducted.

Simple regression analyses were performed to assess the relationship between the GIs for all four potato varieties (fresh boiled and cold test meals) and the physicochemical data (percent amylose, RDS, SDS, and RS) received from AAFC.

Differences and relationships were considered significant if two-tailed P ≤ 0.05. Analysis was done using SPSS Statistics version 17.0.

4.2 Study 2

4.2.1 Overview

During study 2, two varieties (harvested in fall 2009) from study 1 were re-tested. The results of study 1 showed that the GI of these two varieties were affected by cooking and
cooling differently, and thus potatoes were re-investigated to confirm these different effects. This study took place from December 2009-January 2010.

4.2.2 Subjects

Ten healthy subjects (n= five females and five males) with a mean age of 42.3± 3.3 years, mean height of 167.5± 3.2 cm, mean weight of 75.4± 3.0 kg, and mean BMI of 26.9±0.4 kg/m² participated in this study. Subjects in study 2 met the same inclusion criteria, as defined in section 4.1.2. All subjects provided written informed consent before participation. The Research Ethics Board at the University of Toronto granted ethics approval.

4.2.3 Test Meals

Two of the four potato varieties tested during study 1 (varieties 2 and 3) were re-tested during this study. These potatoes however, were harvested during the 2009 growing season. Variety 2 was grown in the same location as the potatoes for study 1 (Vauxhall, Alberta) but were harvested earlier, at 100 days vs. at maturity (120 days). Variety 3 was grown in Guelph, Ontario, while the variety 3 potatoes tested in study 1 were grown in Fredericton, New Brunswick. Both varieties were stored as described in section 4.1.3, but only variety 2 was sprout inhibited.

All ten subjects tested both variety 2 and 3. Preparation methods were the same as those explained in section 4.1.3; however, potatoes were not served to subjects reheated. Each variety was served fresh boiled and cold. Each subject thus consumed four potato test meals.
Subjects were not required to consume a test meal of baked potato chips, as there was only one group of subjects in study 2. Each subject also consumed a test meal of white bread, on two separate occasions, serving as the reference food.

For each variety, total carbohydrate and fibre content was determined by proximate analysis using the methods explained in section 4.1.3, to determine the weight of each potato variety required to feed 50 g of available carbohydrate (Table 3). The composition of the reference white bread was predetermined using proximate analysis, as in study 1 (Table 3).

4.2.4 Protocol

The protocol for this study was similar to that described in section 4.1.4 for study 1. The only difference being subjects were required to visit the lab on six separate occasions (4 potato tests and 2 white bread tests) opposed to ten. Like study 1, all subjects consumed the test meals in random order.

4.2.5 Analytical Methods

Analytical methods performed during this study were the same as those described in section 4.1.5 for study 1. No physicochemical data was received from AAFC for the potatoes in study 2.

4.2.6 Calculation of Glycemic Index

The GI for the potato meals tested during this study, were calculated as described in section 4.1.6 for study 1.
**Table 3.** Test meal composition for Study 2

<table>
<thead>
<tr>
<th>Test Meals</th>
<th>Weight (g)</th>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Total CHO (g)</th>
<th>Fibre (g)</th>
<th>Available CHO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>291</td>
<td>242</td>
<td>0</td>
<td>5.5</td>
<td>54.9</td>
<td>4.9</td>
<td>50</td>
</tr>
<tr>
<td>Variety 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>250</td>
<td>235</td>
<td>0</td>
<td>4.8</td>
<td>54</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>White bread&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104</td>
<td>245</td>
<td>1.7</td>
<td>7.5</td>
<td>52.8</td>
<td>2.8</td>
<td>50</td>
</tr>
</tbody>
</table>

Abbreviations; CHO, carbohydrate

<sup>a</sup>Proximate analysis performed by Agri-Food Laboratories, Guelph (Moisture: AOAC 930.15 (not shown), Protein: AOAC 990.03, Ash: AOAC 942.05 (not shown), Fat: AOAC 920.39, Carbohydrate: by difference); Analysis for Total Dietary Fibre performed by Maxxam Analytics, Mississauga (Total Dietary Fibre: AOAC 993.43, 985.29).

<sup>b</sup>Typical portion size, actual weight varied slightly due to moisture variations.
4.2.7 Statistical Analysis

Results are expressed as means±SEM. Blood glucose profiles, AUC and GI values for each variety (2 and 3) and treatment (fresh boiled and cold) were subjected to ANOVA as described in section 4.1.7.

Additionally, the GI values from study 1 for variety 2 and 3 (fresh boiled and cold) were included in the analysis to investigate the effects of year of harvest on the GI, as potatoes tested in study 1 were harvested in Fall of 2008, and potatoes for study 2 were harvested Fall 2009. GI values from both years were subjected to ANOVA examining for the main effects of potato variety (2 and 3), treatment (fresh boiled and cold), and year of harvest (2008 vs. 2009), and the year×variety, variety×treatment, year×treatment and year×variety×treatment interactions. If any significant interactions were identified ANOVA was used to look at differences in one factor across all levels of another factor, or factors in the case of a three-way interaction. After demonstration of significant heterogeneity, the significance of the differences between GI values was assessed using Bonferroni method for multiple comparisons. Differences were considered significant if two-tailed p ≤ 0.05. Analysis was done using SPSS Statistics version 17.0.

4.3 Study 3

4.3.1 Overview

During study 3, two varieties (harvested in fall 2009) from study 1 were re-tested, one of these varieties being the same variety that was re-tested in study 2. The results of study 1 showed that the GIs of both varieties were affected by cooking and cooling processes
differently so study 3, not only looked to confirm these results but to investigate if the differences between varieties could be explained by slowly or malabsorbed starch. This study took place from March-April 2010.

### 4.3.2 Subjects

Nine of the ten subjects who participated in study 2 also volunteered for study 3. One of the subjects was unable to continue, as he was having difficulty consuming the potato meals; therefore, an additional subject was recruited. Subject characteristics were as follows; four females and six males with a mean age of 43.8± 2.7 years, mean height of 169.3± 3.1 cm, mean weight of 76.8 ± 3.0 kg, and a mean BMI of 26.8± 0.4 kg/m². The new subject was screened, while the other subject’s information was only verified. All subjects met inclusion criteria as defined in section 4.1.2 for study 1. Subjects provided written informed consent before participation and the Research Ethics Board at the University of Toronto granted ethics approval.

### 4.3.3 Test Meals

Variety 1 potatoes were re-tested during this study and where harvested during the 2009 growing season. Variety 1 potatoes for study 3 were grown in Fredericton, New Brunswick (2009), while variety 1 potatoes tested in study 1 where grown in Vauxhall, Alberta (2008). Variety 1 potatoes from both study 1 and 3, were harvested in different years but had a similar harvest date. These potatoes were also sprout inhibited and stored as described in section 4.1.3. Variety 3 was tested again during study 3; but these potatoes were grown in Fredericton, New Brunswick as in study 1. Although study 2 and 3 were conducted during the same harvest year (2009), the variety 3 potatoes grown in
Guelph (study 2) were not sprout inhibited. Since study 3 was planned for several months after harvest, sprout inhibited potatoes were required for a prolonged shelf life. This is why variety 3 potatoes from Fredericton were used, as they had been sprout inhibited prior to shipment. The harvest dates for variety 3 were the same across all three studies, and the potatoes were stored as described in section 4.1.3.

All ten subjects tested both variety 1 and 3. Preparation methods were the same as those explained in section 4.2.3 with each subject consuming four potato test meals. Similarly, to study 2 all subjects consumed a test meal of white bread, on two separate occasions. One of the bread test meals also included 20 ml of a lactulose solution (Ratio-Lactulose 667mg/ml) which provided 10g of lactulose. This was dissolved in 100ml of water. Lactulose represented a known amount of malabsorbed carbohydrate. This allowed for calibration of the amount of H₂ produced (method of collection explained below) by each subject per gram of malabsorbed carbohydrate, estimating semi-quantitatively the amount of carbohydrate malabsorbed after each of the other test meals. Table 4 includes the test meal composition for study 3, as determined by proximate analysis described in section 4.1.3.

4.3.4 Protocol

Subjects visited the lab on 6 separate occasions (4 potato test meals, 1 white bread and 1 white bread + lactulose test meal), consuming the test meals in random order. Each test lasted for 8 hours (480 min). Over the first 2 hours, a test meal was consumed and blood samples were obtained exactly as described above in section 4.1.4. Once the 2 hour (120 min) blood sample had been taken subjects remained seated quietly, until 4 hours (240
Table 4. Test meal composition for Study 3

<table>
<thead>
<tr>
<th>Test Meals</th>
<th>Weight (g)</th>
<th>Energy (kcal)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Total CHO (g)</th>
<th>Fibre (g)</th>
<th>Available CHO (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>235</td>
<td>237</td>
<td>0.24</td>
<td>5.4</td>
<td>53.5</td>
<td>3.5</td>
<td>50.0</td>
</tr>
<tr>
<td>Variety 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>301</td>
<td>250</td>
<td>0.30</td>
<td>6.3</td>
<td>55.4</td>
<td>5.4</td>
<td>50.0</td>
</tr>
<tr>
<td>White bread&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104</td>
<td>245</td>
<td>1.7</td>
<td>7.5</td>
<td>52.8</td>
<td>2.8</td>
<td>50.0</td>
</tr>
</tbody>
</table>

Abbreviations; CHO, carbohydrate

<sup>a</sup>Proximate analysis performed by Agri-Food Laboratories, Guelph (Moisture: AOAC 930.15 (not shown), Protein: AOAC 990.03, Ash: AOAC 942.05 (not shown), Fat: AOAC 920.39, Carbohydrate: by difference); Analysis for Total Dietary Fibre performed by Maxxam Analytics, Mississauga (Total Dietary Fibre: AOAC 993.43, 985.29).

<sup>b</sup>Typical portion size, actual weight varied slightly due to moisture variations.
min), where they took another finger-prick blood sample and consumed a standard lunch meal provided (described below). Subjects consumed the lunch meal within 15 minutes and additional blood samples were taken at 255, 270, 285, 300, 330, 360 minutes after starting to eat their morning test meal. Blood glucose was measured after the standard lunch meal, to investigate the second meal effect of the potato test meals on the blood glucose response of a meal served later.

The standard lunch consisted of a cheese, tomato, lettuce and turkey sandwich (2 slices of Dempster’s white bread, 20g of cheddar marble cheese (Selection brand), 30g hot house tomato, 10g romaine lettuce, 60g oven roasted turkey breast (Selection brand), and 1 tbsp Helman’s ½ The Fat Mayonnaise), a choice of either 250ml apple juice (Rougemont McIntosh apple juice) or 250ml orange juice (Tropicana No Pulp 100% orange juice), and two Oreo cookies (choice of either Golden Oreo or Original Oreo). One subject was a vegetarian and requested no turkey, and another subject requested no cheese. Food choices and amounts determined at the first visit remained consistent for the other five test days. The lunch provided approximately 606 kcal, 25.4 g protein, 19.5g fat, 82.5g carbohydrate and 2.1g fibre. The macronutrient content of each food choice is presented in Table 5.

In addition to blood samples, breath samples were collected fasting (2 samples) and at hourly intervals after the start of the test meal for 8 hours. Thus each subject collected a total of 10 breath samples per test day. Expired air was collected into 10 ml evacuated glass tubes using an EasySampler device (Quintron Instruments, Milwaukee, WI, USA) and stored at room temperature. After the last blood sample (360 minutes), subjects had
Table 5. Macronutrient composition of standard lunch for Study 3<sup>a</sup>

<table>
<thead>
<tr>
<th>Possible Choices</th>
<th>Serving</th>
<th>Kcal</th>
<th>Fat</th>
<th>Protein</th>
<th>CHO</th>
<th>Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dempsters White Bread</td>
<td>2 slices (71g)</td>
<td>170kcal</td>
<td>1.5g</td>
<td>6g</td>
<td>34g</td>
<td>1g</td>
</tr>
<tr>
<td>Oven Roasted Turkey Breast</td>
<td>3 slices (60g)</td>
<td>60kcal</td>
<td>1g</td>
<td>12g</td>
<td>2g</td>
<td>0g</td>
</tr>
<tr>
<td>Rougemont McIntosh Apple Juice</td>
<td>250ml</td>
<td>110kcal</td>
<td>0g</td>
<td>0g</td>
<td>27g</td>
<td>0g</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tropicana No Pulp 100% Orange Juice</td>
<td>250ml</td>
<td>110kcal</td>
<td>0g</td>
<td>2g</td>
<td>27g</td>
<td>0g</td>
</tr>
<tr>
<td>Golden Oreo Cookie</td>
<td>2 cookies (24g)</td>
<td>120kcal</td>
<td>5.0g</td>
<td>1g</td>
<td>17g</td>
<td>0g</td>
</tr>
<tr>
<td>OR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oreo Original Cookie</td>
<td>2 cookies (24g)</td>
<td>120kcal</td>
<td>5.0g</td>
<td>1g</td>
<td>17g</td>
<td>1g</td>
</tr>
<tr>
<td>Helman’s ½ the fat Mayonnaise</td>
<td>1 tbsp</td>
<td>45kcal</td>
<td>5g</td>
<td>0g</td>
<td>1g</td>
<td>0g</td>
</tr>
<tr>
<td>Tomato</td>
<td>2 slices (30g)</td>
<td>6 kcal</td>
<td>0g</td>
<td>0.3g</td>
<td>1.2g</td>
<td>0.4g</td>
</tr>
<tr>
<td>Romaine Lettuce</td>
<td>1 leaf (10 g)</td>
<td>2 kcal</td>
<td>0g</td>
<td>0.1g</td>
<td>0.3g</td>
<td>0.2</td>
</tr>
<tr>
<td>Cheddar Marble Cheese-Selection</td>
<td>1 slice (20g)</td>
<td>80kcal</td>
<td>7g</td>
<td>5g</td>
<td>0g</td>
<td>0g</td>
</tr>
</tbody>
</table>

Abbreviations; CHO, carbohydrate

<sup>a</sup>Nutritional information for each food was obtained from its nutrition fact table. Lettuce and tomato nutritionals obtained from the Canadian Nutrient File (Health Canada, 2009).
the option to either remain at the lab or take the EasySampler home to collect their last two breath samples. If they decided to go home they were instructed to bring the tubes back to the lab on their next scheduled visit.

4.3.5 Analytical Methods

Finger-stick blood samples from both the morning and lunch meals were analyzed for glucose as described for study 1 in section 4.1.5. Two out of 825 (0.24%) blood samples were recorded as missing secondary to blood cloting during storage. Missing data were imputed by averaging the preceding and following values.

Breath samples were analyzed for carbon dioxide, H₂, and methane content using gas chromatography (Microlyzer Gas Analyzer, model SC; Quintron Intruments) at the University of Toronto. Very high carbon dioxide concentrations were present in 5 of the 600 breath samples (0.83%); these were considered poor samples and data were entered as missing. Missing data were imputed by interpolating from preceding and following values. If the last breath sample (480 min) was missing, the 420 min value was inserted.

4.3.6 Calculation of Glycemic Index

Blood glucose data collected during the morning session of study 3 were used to calculate the GI of the potato meals, as described for study 1 in section 4.1.6.

4.3.7 Statistical Analysis

Results are expressed as means±SEM. AUCs were calculated separately for 0-120 min (blood glucose response to morning test meals) and 240-360 min (blood glucose response
to a standard lunch). The GI values of the potatoes were calculated as described for study 1 using the AUC values for 0-120 min. Blood glucose profiles (0-120 min and 240-360 min), AUC (0-120 min and 240-360 min) and GI values for each variety (1 and 3) and treatment (fresh boiled and cold) were subjected to ANOVA as described in section 4.1.7.

The GI values from study 1 for variety 1 and 3 (fresh boiled and cold) were included in the analysis to investigate the effects of year of harvest on the GI. The statistical analysis described for study 2 in section 4.2.7 were the procedures used.

Breath hydrogen profiles for each variety and treatment were subjected to ANOVA, similar to the procedures described in 4.1.7 for the blood glucose profiles. The total hydrogen produced by each subject over each test day was calculated by summing the hydrogen concentration from baseline (defined as the lowest value in the first 3 hours) to 8 hrs. To evaluate the differences between test meals, total hydrogen data was subjected to ANOVA, as described in section 4.1.7 for the blood glucose AUC. In a separate analysis, hydrogen profiles after lactulose were compared to each potato meal profile using a two factor ANOVA (time× test meal) while total hydrogen produced after lactulose was compared to each of the potato meals and white bread using a one factor (test meal) ANOVA.

Total $H_2$ per gram of lactulose, $L$, was calculated for each subject as $L = (BL-B)/10$ g, where $BL$ is total $H_2$ after the white bread plus lactulose meal and $B$ is total $H_2$ after white bread alone. The amount of malabsorbed carbohydrate in each potato test meal was calculated for each subject as $P/L$, where $P$ is total $H_2$ after the potato test meal and $L$ is total $H_2$ per gram of lactulose. Differences between test meals in terms of malabsorbed
carbohydrate were assessed using ANOVA, as described for blood glucose AUCs and total hydrogen. The amount of malabsorbed carbohydrate in white bread, was compared to each of the potato meals using a one factor ANOVA.

After demonstration of significant heterogeneity, the significance of differences between means was assessed using the Bonferroni method to adjust for multiple comparisons. If some results appeared not to be normally distributed, they were subjected to log-transformation prior to statistical analysis. All differences were considered significant if two-tailed $P \leq 0.05$. In keeping consistent with study1 and study 2, all analysis for study 3 was conducted using SPSS Statistics version 17.0.
Chapter 5

Results

5.1 Study 1

The GI for baked potato chips was the same for both subject group A and group B, 72.3 ± 6.1 and 71.2 ± 8.2 respectively (F= 0.279, p=0.929). This provides validity for between group comparisons of the GI for the different potato varieties.

Variety 2 began to spoil halfway through the study, as a result 6 tests involving this variety were unable to be completed including; 3 fresh boiled, 2 reheated and 1 cold test. Missing data were interpolated based on a method described by Cochran & Snedecor (1980).

Blood glucose response elicited by fresh boiled, reheated and cold potatoes for varieties 1, 2, 3 and 4 harvested in 2008 are shown in Figure 1. ANOVA could not identify a time × variety interaction (F=1.5, p=0.99) or a time × treatment × variety interaction (F=1.2, p=0.28) but a significant time × treatment interaction (F=7.5, p=<0.001) for each variety was identified. Fresh boiled potatoes at 15 min elicited a significantly greater blood glucose response than cold potatoes for variety 2, while in variety 1 fresh boiled potatoes elicited a greater response at both 15 and 30 min. For variety 3, reheated and fresh boiled treatments both elicited a significantly greater response than cold potatoes at 15, 45 and 60 min; at 30 min only the fresh boiled treatment was greater than the cold. Finally, for variety 4, at 15, 45 and 60 min fresh boiled potatoes elicited a significantly greater response than cold potatoes. At 30 min both reheated and fresh boiled treatments elicited
Figure 1. Glucose response elicited by fresh boiled, reheated and cold potatoes for varieties 1, 2, 3 and 4 harvested in 2008. Values are means ± SEM (n=10). There was a significant time x treatment interaction for each variety, F=7.5, p<0.001. Significant differences; fresh boiled vs. cold treatments denoted by “a”; reheated vs. cold treatments denoted by “b”; fresh boiled vs. reheated treatments denoted by “c”. All differences significant at P<0.05.
a greater response than cold potatoes, and fresh boiled potatoes had a higher glucose 
concentration than reheated potatoes at this time point. All differences were significant at 
P<0.05.

There was no main effect of potato variety for the mean blood glucose AUC (F=1.4, 
p=0.241) but a main effect of treatment (F=7.0, p= 0.001), with fresh boiled and reheated 
potatoes having significantly greater AUCs than cold potatoes (182.5±27.6 and 
181.7±26.7 vs. 123.2±21.7). There was no treatment ×variety interaction for the AUC of 
the potato meals (F=1.1, p=0.387) (Table 6).

GI values for all potato varieties (harvested 2008) are shown and compared to the GI of 
white bread in Table 6. The GI of variety 3 cold (40.3±5.3) was the only potato test meal 
that differed significantly from that of white bread (GI= 71). Three of the twelve potato 
meals (variety 2, 3, 4 cold) could be classified as low GI (GI ≤55). Five potato meals had 
an intermediate GI (GI =55-69), while only four were classified as high GI (GI= ≥ 70) 
(Table 6). ANOVA, showed no main effect of potato variety (F =0.9, p=0.42), but a 
significant main effect of treatment (F = 15.1, p<0.001), with cold potatoes having a 
lower GI than freshly boiled and reheated potatoes (48.7±6.4 vs. 67.9±6.4 and 70.2±5.1, 
respectively) (Table 6). There was however, a significant treatment× variety interaction 
(F=2.6, p=0.024), with cooling significantly reducing the GI of variety 3 and 4 and not 
significantly reducing the GI of variety 1 and 2 (Table 6 and Figure 2). Figure 3, further 
emphasizes the differences between varieties upon cooling a fresh boiled potato, as the 
38.7±8.5 unit reduction upon cooling variety 3 was significantly greater than the 
reduction in GI upon cooling varieties 1 and 2 (4.5±7.4 and 6.2±6.9 respectively) 
(P<0.05).
Table 6. AUC and GI values for varieties 1,2,3, and 4 harvested in 2008, served to subjects (n=10) fresh boiled, reheated and cold.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean for Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>Fresh Boiled</td>
</tr>
<tr>
<td>AUC (mmol×min/L)</td>
<td></td>
</tr>
<tr>
<td>Variety 1</td>
<td>158.8±27.8</td>
</tr>
<tr>
<td>Variety 2</td>
<td>140.4±12.9</td>
</tr>
<tr>
<td>Variety 3</td>
<td>230.8±37.6</td>
</tr>
<tr>
<td>Variety 4</td>
<td>200.1±32.1</td>
</tr>
<tr>
<td>Mean for Treatment</td>
<td>182.5±27.6</td>
</tr>
</tbody>
</table>

Glycemic Index

| Variety         | 59.8±5.2         | 75.3±5.3 | 55.3±6.9 | 63.5±5.8 |
| Variety 2       | 61.2±3.9         | 69.1±5.3 | 55.0±6.5 | 61.8±5.2 |
| Variety 3       | 79.1±8.8         | 78.5±4.3 | 40.3±5.3 | 66.0±6.1 |
| Variety 4       | 71.6±7.8         | 57.8±5.3 | 44.1±6.7 | 57.8±6.6 |
| Mean for Treatment | 67.9±6.4         | 70.2±5.1 | 48.7±6.4 |

Values are means ± SEM

^b GI values not sharing the same letter superscripts differ significantly within each row (P<0.05).

^y GI values of potato meals that differ significantly from the GI of white bread (GI= 71).
Figure 2. GI of fresh boiled, reheated and cold potatoes for varieties 1, 2, 3 and 4. Values are means ± SEM (n=10). A significant treatment x variety interaction existed (F=2.6, p=0.024). Bars with different letters, within each variety differ significantly (P<0.05).
Figure 3. Reduction in the GI upon cooling fresh boiled potatoes. Each bar represents the difference between the GI of fresh boiled and cold potatoes for each variety. Values are means ± SEM (n=10). The reduction in the GI upon cooling variety 3 was significantly greater than the reduction in variety 1 and 2 (P<0.05). Bars with different letters, differ significantly (P<0.05).
There was a significant positive correlation between the GI of fresh boiled potatoes and % SDS ($r=0.982$, $p=0.02$). Although GI of fresh boiled potatoes was negatively correlated with both % RDS ($r=-0.866$, $p=0.13$) and % Amylose ($r=-0.823$, $p=0.17$) these relationships did not reach significance (Figure 4). There was a significant negative correlation between the GI of cold potatoes and % SDS ($r=-0.951$, $p=0.049$), but the positive correlations between the GI of cold potatoes and % RDS ($r=0.883$, $p=0.12$) and % Amylose ($r=0.825$, $p=0.17$), were not significant (Figure 5). Finally the % RS in potatoes were not correlated with the GI of either fresh boiled ($r=0.457$, $p=0.54$) or cold potatoes ($r=-0.527$, $p=0.47$) (Figures 4 & 5).

5.2 Study 2

Blood glucose response elicited by fresh boiled and cold potatoes for varieties 2 and 3, harvested in 2009 are shown in Figure 6. A significant time× treatment× variety interaction existed, ($F= 5.4$, $p= 0.005$). At 30 minutes, the blood glucose concentration after variety 3 fresh boiled was significantly greater than after variety 3 cold ($p<0.001$) and there was no difference between treatments for variety 2 at this time point. There were no significant differences between treatments for any of the other time points. In terms of mean blood glucose AUC for variety 2 and 3 (harvested 2009), there was no main effect of treatment ($F=4.1$, $p=0.059$) but a significant main effect of potato variety ($F=9.9$, $p=0.005$), with variety 3 having a significantly higher AUC than variety 2, 141±14 vs. 93±13, respectively. A significant treatment ×variety interaction existed ($F=8.8$, $p=0.008$), as cooling significantly reduced the AUC in variety 3, and had no effect on the AUC of variety 2 (Table 7).
Figure 4. Correlations between the GI of fresh boiled potatoes and: A % RDS; B % SDS; C % RS; and D % Amylose.
Figure 5. Correlations between the GI of cold potatoes and: A % RDS; B % SDS; C % RS; and D % Amylose.
Figure 6. Glucose response elicited by fresh boiled and cold potatoes for varieties 2 and 3 harvested in 2009. Values are means ± SEM (n=10). There was a significant time ×treatment ×variety interaction, F= 5.4, p= 0.005. Significant differences; fresh boiled vs. cold treatments for variety 3 denoted by “a” (p<0.001).
Table 7. AUC and GI values for varieties 2 and 3 harvested in 2009, served to subjects (n=10) fresh boiled and cold

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Mean for Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Boiled</td>
<td>Cold</td>
</tr>
<tr>
<td>AUC (mmol x min/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety 2</td>
<td>87±12</td>
<td>98±13</td>
</tr>
<tr>
<td>Variety 3</td>
<td>168±14⁺</td>
<td>113±14⁻</td>
</tr>
<tr>
<td>Mean for Treatment</td>
<td>128±13</td>
<td>106±14</td>
</tr>
<tr>
<td>Glycemic Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety 2</td>
<td>37.8±5.5</td>
<td>40.6±5.0</td>
</tr>
<tr>
<td>Variety 3</td>
<td>72.5±6.1⁺</td>
<td>48.6±6.9⁻</td>
</tr>
<tr>
<td>Mean for Treatment</td>
<td>55.2±5.8</td>
<td>44.6±6.0</td>
</tr>
</tbody>
</table>

Values are means ± SEM

⁺⁻ Values not sharing the same letter superscripts differ significantly within each row (P<0.05).

⁺⁻ Mean AUC and GI for variety 2 differ significantly from mean of variety 3 (P<0.05).
The GI values for variety 2 and 3 harvested in 2009, are listed in Table 7. Variety 3 cold and variety 2, fresh boiled and cold had low GI values (GI ≤55), while variety 3 fresh boiled had a high GI (GI ≥70). Consistent with the AUC results there was no main effect of treatment (F=4.3, p= 0.054), but a main effect of variety (F=10.3, p=0.005), with variety 3 having a significantly higher GI than variety 2, 60.5±6.5 vs. 39.2±5.3, respectively. There was a significant treatment× variety interaction (F=6.8, p=0.018), as the GI for variety 3 fresh boiled was significantly greater than its GI cold, while there was no difference between fresh boiled and cold potatoes for variety 2 (Table 7).

Figure 7 shows the GI’s for variety 2 and 3, harvested during 2008 and 2009. There was a significant year of harvest ×variety interaction (F=5.1, p=0.027), as the GI of variety 2 differed across year of harvest while variety 3 virtually had the same GI two harvest years in a row (Figure 8A). A significant treatment ×variety (F=11.6, p=0.001) interaction also existed, indicating that cooling significantly reduced the GI of variety 3, while having no effect on the GI of variety 2 (Figure 8B). The year of harvest ×treatment interaction was not significant (F=1.87, p=0.176) and there was no significant year of harvest ×treatment ×variety interaction (F=0.114, p=0.736).

5.3 Study 3

5.3.1 Glycemic responses and GI

Blood glucose responses elicited by fresh boiled and cold potatoes for varieties 1 and 3, harvested in 2009 are shown in Figure 9. There was no treatment × variety (F=0.99, p=0.756), time × variety (F=1.68, p=0.203) and no treatment × time × variety (F=1.38,
Figure 7. GI of fresh boiled and cold potatoes for varieties 2 and 3 harvested in 2008 and 2009. Values are means ± SEM (n=10).
Figure 8. A: Each bar represents the average GI of the fresh boiled and cold potato for that year (2008 or 2009) and that variety (2 or 3). A year of harvest × variety interaction existed (F=5.1, p=0.027), the GI of variety 2 differed significantly between years but no difference in GI between years for variety 3. B: Each bar represents the average GI of the 2008 and 2009 potatoes for that treatment (fresh boiled or cold) and that variety (2 or 3). A treatment × variety interaction existed (F=11.6, p=0.001), cooling significantly reduced the GI of variety 3, and had no effect on variety 2. Values expressed as mean ± SEM (n=2). Bars with different letters, within each graph differ significantly (P<0.05).
**Figure 9.** Glucose response elicited by fresh boiled and cold potatoes for varieties 1 and 3, harvested in 2009 taken at breakfast (0 min) followed by a standard lunch taken at 240 min. Values are means ± SEM (n=10). There was a significant time× treatment interaction (F=11.4, p<0.001) after breakfast (0 -120 min) and a significant main effect of treatment (F=5.6, p=0.029) after the standard lunch (240-360 min). Significant differences; fresh boiled vs. cold treatments for variety 1 denoted by “a”; fresh boiled vs. cold treatments for variety 3 denoted by “b (P<0.05).
p=0.292) interactions for glucose concentrations after the potato test meals (0-120 min). However, there was a significant time × treatment interaction (F= 11.4, p<0.001) (Figure 9). At 15 minutes blood glucose was significantly higher after variety 3 fresh boiled than after variety 3 cold. Blood glucose continued to rise and at 30 minutes, fresh boiled potatoes for both variety 1 and 3 were significantly higher than cold potatoes. The differences in peak rise (45 min) was significant, as both variety 1 and 3 fresh boiled potatoes had significantly higher glucose concentrations than cold potatoes. Finally, blood glucose at 60 min was significantly higher after fresh boiled potatoes for both variety 1 and 3 than for cold potatoes. All difference were significant at P<0.05. For the glucose concentrations after the standard lunch meal (240-360 min) there was no time × treatment (F=0.46, p=0.826), variety × time (F=0.57, p=0.746), treatment × variety (F=.435, p=.518) and no treatment × time × variety (F=1.65, p=0.21) interactions. A main effect of treatment (F=5.6, p=0.029) however was identified, with fresh boiled potatoes having significantly lower glucose concentrations than cold potatoes (5.52±0.13 vs. 5.71±0.11, respectively) (Figure 9). Although a time × treatment interaction was not identified after the lunch meal (240-360 min) the effects of treatment were investigated at each time point. According to this secondary analysis, at 240 min (before start of lunch meal), the blood glucose concentration for variety 3 fresh boiled was significantly lower than for variety 3 cold potatoes and at 255 min (15 min after the start of lunch), variety 1 fresh boiled had a significantly lower blood glucose concentration than variety 1 cold potatoes. All differences were considered significant at P<0.05.

Concerning the mean 0-120 min AUC, there was no main effect of variety (F=1.12, p=0.307) or treatment × variety (F=2.06, p=0.168). A main effect of treatment (F=20.4,
p<0.001) was identified however, as the AUC after cold potato meals, had a significantly lower AUC than after fresh boiled potato meals (122±15 vs. 203±23, respectively). Specifically, variety 1 cold was 47% lower than for variety 1 fresh boiled while; variety 3 cold had an AUC 31% lower than variety 3 fresh boiled. These differences were significant at (P<0.05) (Table 8). In terms of the AUC for the standard lunch (240-360 min), there was no main effect of treatment (F=0.52, p=0.482) or variety (F=0.19, p=0.668) and no interaction (F=0.001, p=0.970) was found (Table 8).

The GI values for variety 1 and 3 harvested in 2009, are listed in Table 8. Variety 1 and 3 cold were measured as low GI (GI ≤ 55). Fresh boiled, variety 1 and 3 potatoes were classified as high (GI ≥ 70) (Table 8). As with the mean 0-120 min AUC, ANOVA showed a main effect of treatment (F=26.4, p<0.001), with fresh boiled potatoes having a significantly higher GI than the cold potatoes (78.2±7.9 vs. 49.3±5.7, respectively). No treatment× variety interaction was found (F=0.792, p=0.385), and thus both variety 1 and 3 fresh boiled potatoes had significantly greater GIs than variety 1 and 3 cold. A main effect of variety was also not identified (F=0.129, p=0.713) (Table 8).

Upon factoring in the year of harvest (2008 vs. 2009) into the analysis a significant year of harvest× variety× treatment (F=5.3, p=0.025) interaction was identified. Where the GI of variety 1 was significantly reduced by cooling during the 2009 year of harvest and not reduced during the 2008 year of harvest, while the GI of variety 3 was reduced upon cooling, two harvest years in a row (Figure 10).

5.3.2 Breath Hydrogen and Malabsorbed Carbohydrate

There was no significant time × treatment (F=1.84, p=0.173), time × variety (F=0.384,
Table 8. AUC (0-120 min and 240-360 min) and GI values for varieties 1 and 3# harvested in 2009, served to subjects (n=10) fresh boiled and cold

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Mean for Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Boiled</td>
<td>Cold</td>
</tr>
<tr>
<td>AUC (mmol x min/L)- 0-120 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety 1</td>
<td>228±32a</td>
<td>121±17b</td>
</tr>
<tr>
<td>Variety 3</td>
<td>178±14a</td>
<td>123±13b</td>
</tr>
<tr>
<td>Mean for Treatment</td>
<td>203±23a</td>
<td>122±15b</td>
</tr>
<tr>
<td>AUC (mmol x min/L)- 240-360 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety 1</td>
<td>228±26</td>
<td>218±28</td>
</tr>
<tr>
<td>Variety 3</td>
<td>217±17</td>
<td>205±17</td>
</tr>
<tr>
<td>Mean for Treatment</td>
<td>223±22</td>
<td>212±23</td>
</tr>
<tr>
<td>Glycemic Index (GI= 100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety 1</td>
<td>82.2±6.8a</td>
<td>48.3±5.5b</td>
</tr>
<tr>
<td>Variety 3</td>
<td>74.2±9.0a</td>
<td>50.3±5.9b</td>
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<tr>
<td>Mean for Treatment</td>
<td>78.2±7.9a</td>
<td>49.3±5.7b</td>
</tr>
</tbody>
</table>

Values are means± SEM

# Variety 3 had a different harvest location than study 2, but the same harvest year (2009)

ab Mean values not sharing the same letter superscripts differ significantly within each row (P<0.05).
**Figure 10.** GI of fresh boiled and cold potatoes for varieties 1 and 3, harvested in 2008 and 2009. Values are means ± SEM (n=10). A significant year of harvest × variety × treatment interaction existed (F=5.3, p=0.025), with the effect of cooling on the GI dependent upon the year of harvest and potato variety. Bars with different letters, within each variety differ significantly (P<0.05).
p=0.908), treatment × variety (F=0.722, p=0.407) and no treatment× time× variety
(F=0.629, p=0.314) interactions for breath hydrogen concentrations (Figure 11). A main
effect of treatment however was found (F=4.6, p=0.045) with cold potatoes producing
significantly greater hydrogen concentrations than fresh boiled potatoes (7.8±1.8 vs.
4.5±0.82, respectively). Although a time × treatment interaction was not identified for
hydrogen concentrations the effects of treatment were investigated at each time point.
According to this secondary analysis at 420 min and 480 min hydrogen concentrations
for variety 1 cold, were greater than variety 1 fresh boiled (P<0.05).

When the hydrogen concentrations after lactulose were compared to the potato meals,
ANOVA identified significant differences at 6 of the 9 time points. For example, at 180
min and 240 min hydrogen concentrations of lactulose were significantly greater than all
four potato meals. Hydrogen for lactulose at 120 min was significantly greater than
variety 1 fresh boiled and variety 1 cold. At 300 min variety 3 cold was the only potato
meal that had significantly lower hydrogen concentrations than lactulose. Variety 1 fresh
boiled, variety 1 cold and variety 3 fresh boiled had lower hydrogen levels than lactulose
at 360 min. Finally, at 420 min lactulose was greater than variety 1 fresh boiled and
variety 3 fresh boiled. All differences were considered significant at P<0.05.

The values for total breath hydrogen were not normally distributed thus data was logged
for statistical analysis. However, absolute values are presented in Table 9 for all test
meals. There was a main effect of treatment for total hydrogen (F=6.4, p=0.021), as total
hydrogen after cold potatoes was significantly greater than that after fresh boiled
potatoes, 61.2 ± 19.4 ppm and 33.9± 9.4 ppm respectively. No main effect of variety
Figure 11. Breath hydrogen responses elicited by fresh boiled and cold potatoes for varieties 1, 3 and lactulose. Values are means ± SEM (n=10). Main effect of treatment for potato test meals (F=4.6, p=0.045) and breath hydrogen concentrations for lactulose differed significantly from potato test meal concentrations (P<0.05). Significant differences: fresh boiled vs. cold treatments for variety 1 denoted by “a”, variety 1 fresh boiled vs. lactulose denoted by “b”, variety 1 cold vs. lactulose denoted by “c”, variety 3 fresh boiled vs. lactulose denoted by “d”, variety 3 cold vs. lactulose denoted by “e”. All differences significant at P<0.05.
Table 9. Total breath hydrogen response after consumption of potato, white bread and lactulose test meals (n=10).

<table>
<thead>
<tr>
<th></th>
<th>Treatment</th>
<th>Mean for Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh boiled</td>
<td>Cold</td>
</tr>
<tr>
<td><strong>Total Breath Hydrogen (ppm)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variety 1</td>
<td>28.6± 6.7 \textsuperscript{y}</td>
<td>41.30±7.7 \textsuperscript{yp}</td>
</tr>
<tr>
<td>Variety 3</td>
<td>39.2± 12.0 \textsuperscript{y}</td>
<td>81.0± 31.1 \textsuperscript{yp}</td>
</tr>
<tr>
<td><strong>Mean for Treatment</strong></td>
<td>33.9±9.4\textsuperscript{a}</td>
<td>61.2±19.4\textsuperscript{b}</td>
</tr>
<tr>
<td>White Bread</td>
<td>19.3 ±5.0 \textsuperscript{y}</td>
<td></td>
</tr>
<tr>
<td>Lactulose\textsuperscript{#}</td>
<td></td>
<td>233.4±34.9</td>
</tr>
</tbody>
</table>

Data not normally distributed, logged for statistical analysis

Values are means ±SEM

* Calculated by summing the hydrogen concentration from baseline (defined as the lowest value in the first 3 hours) to 8 hrs

\# Consumed with white bread test meal, total hydrogen produced for lactulose, determined by subtracting total hydrogen produced after white bread.

\textsuperscript{y} Means differ significantly from lactulose test meal (P<0.05).

\textsuperscript{p} Means differ significantly from white bread test meal (P<0.05).

\textsuperscript{a,b} Values with different letters differ significantly from each other, within each row (P<0.05).
(F=1.13, p=0.302) nor a treatment x variety interaction (F=0.001, p=0.982) were found.

Upon factoring in white bread and lactulose to the analysis, total hydrogen for white bread and potato meals were all significantly lower than that for lactulose (P<0.05). Variety 1 and 3 cold had significantly greater total hydrogen than after white bread (41.30±7.7 ppm and 81.0±31.1 ppm vs. 19.3±5.0 ppm, respectively) (P<0.05).

Data for malabsorbed carbohydrate after each of the test meals was also not normally distributed and thus logged for statistical analysis. Absolute values are presented in Table 10. There was a main effect of treatment (F=6.38, p=0.021), as the amount of malabsorbed carbohydrate in the cold potato test meals was significantly greater than the amount in the fresh boiled potatoes, 2.5±0.4 g and 1.6±0.3 g, respectively. No main effect of variety (F=1.47, p=0.240) nor a treatment x variety interaction (F=0.001, p=0.982) existed. In comparing the amount of malabsorbed carbohydrate in the potato meals to that of white bread, variety 1 cold and variety 3 cold had significantly greater amounts of malabsorbed carbohydrate (1.8±0.2 g and 3.2±0.9 g vs. 0.8±0.1 g) (P<0.05).
**Table 10.** Amount of malabsorbed carbohydrate (CHO) in potato and white bread test meals (n=10).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean for Variety</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety 1</td>
<td>1.6±0.5</td>
</tr>
<tr>
<td>Malabsorbed CHO (g)</td>
<td>1.8±0.2</td>
</tr>
<tr>
<td>Variety 3</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td></td>
<td>3.2±0.9</td>
</tr>
<tr>
<td>Mean for Treatment</td>
<td>1.6±0.4</td>
</tr>
<tr>
<td></td>
<td>2.5±0.6</td>
</tr>
<tr>
<td>White Bread</td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

Data not normally distributed, logged for statistical analysis

Values are means ±SEM

* Calculated by taking the amount of H₂ produced by each subject per gram lactulose and determining a semi-quantitative amount of carbohydrate malabsorbed after each of the other test meals.

*y* Means differ significantly from white bread test meal (P<0.05).

*a b* Means with different letters differ significantly from each other (P<0.05).
Chapter 6

Discussion

6.1 Study 1

The 2008 *International Tables of Glycemic Index and Glycemic Load Values* reports GI values of fresh potatoes ranging from as low as 23 to as high as 118 on the glucose scale (GI=100), irrespective of variety, cooking or cooling method (Atkinson et al., 2008). The potatoes identified as being cooked then cooled, from the table generally tend to have low to intermediate GIs (23, 56 and 67) however this is not always the case, as one out of the total four cooled potato values reported, had a GI of 88. From forty values (excluding, cooled, canned, French fried, instant and instant mashed potatoes) 28% can be classified with an intermediate GI, while the remaining 72% are classified as high GI (Atkinson et al., 2008). Without considering methodological errors between studies, the distribution of GI values alone from this table warrants the perception of the potato as high GI. With regards to the four potato varieties (12 potato meals) tested in study 1, the majority of the potato meals (67%) were either low or intermediate, and the four potato meals that were classified as high GI all had values less than 80 (Table 6). This is compared to 62% (18/29) of the high values from the 2008 *International Tables* with a GI over 80 (Atkinson et al., 2008).

The potatoes tested in this study generally have lower GI values than the potatoes reported in the 2008 *International Tables* particularly if you consider the mean for each variety or each treatment (Table 6). In addition, there is perception that a boiled or
cooked potato has a GI or a glucose response similar to that of glucose. However, even within the 2008 International Tables, there are very few potatoes with GIs greater than 90. While all of the potato meals tested in study 1 were at least 20 units lower than the GI of glucose, although all, with the exception of variety 3 cold were comparable to the GI of white bread (GI=71). It is fair to say that based on this pilot study perhaps not low, but lower GI potato varieties have been identified, and it is not only the cold potato meals that fit into this category, as the fresh boiled variety 1 and 2 potatoes have GIs of 59.8 and 61.2, respectively. The identification of low GI potatoes during this pilot study suggests that the plant breeders and starch chemists of the BPN may be approaching their goal in breeding potato starch that is more slowly or less absorbed than those varieties available commercially.

The 2008 International Tables reports the majority of its GI values for potatoes from six main studies (Fernandes et al., 2005; Aston et al., 2008; Henry et al., 2005; Leeman et al., 2005; Leeman et al., 2008; Soh & Brand-Miller, 1999). Of these studies, not one investigated interactions between variety and preparation. In other words, they did not determine whether the effects of a particular preparation method (i.e. cooling) on the GI of one variety influenced other varieties similarly. One study by Fernandes et al. (2005) suggested that the effect of preparation method on GI may depend on the variety of potato as cooling and reheating baked Russet potatoes reduced the glycemic response by approximately 30%, whereas there was no effect for California white potatoes. This interaction, however was not tested directly and the white potatoes although a different variety were boiled not baked, thus the differences in glycemic responses between varieties with respect to cooling and reheating could be due to initial preparation method
(i.e. boiled vs. baked) and not due to differences in variety. This interaction likely existed however, as Soh & Brand-Miller. (1999) determined no differences in GI between four different cooking methods (baked, boiled and mashed, boiled and microwaved) in a Pontiac potato. Both of these studies helped with the design for study 1; first to investigate an interaction, and secondly to focus on one cooking treatment. From the six studies listed above, only two investigated the effects of cooling a previously cooked potato (Fernandes et al., 2005; Leeman et al., 2005), and both reported reductions in GI, although not to the same extent. Thus, it seemed important to investigate the effects of cooling and determine if these effects differed between the four varieties that supposedly differed with respect to their physicochemical composition.

As expected the effects of preparation method on the GI differed depending on potato variety. Specifically, cooling lowered, the GI of varieties, 3 and 4 but did not lower the GI in varieties 1 and 2. The difference in growing location between both pairs of varieties may explain some of the differences. Variety 3 and 4, which were harvested in Fredericton, New Brunswick, reacted similarly to all three treatments, as did variety 1 and 2 from Vauxhall, Alberta (Table 6). Different growing conditions and environments have been shown to affect certain physicochemical properties such as granular size, amylose, and phosphorus content of potato starch (Cottrell et al., 1995; Haase & Plate, 1996). These properties to varying degrees have been shown to effect starch hydrolysis (Liu et al., 2007). This may explain some of the similarities noted within growing location. Unfortunately, we were not provided with specific information pertaining to soil composition, average growing temperatures, or weather conditions, and we received limited data concerning each variety’s physicochemical make up. Therefore, it is difficult
to determine which factor or combination of factors may have contributed to a specific starch property. Although, variations in growing environment could definitely be a contributor, the likely culprit is that the genotypes within each pair (variety 1 and 2 vs. variety 3 and 4) are quite similar to each other, while between each pair of varieties the genotypes differ quite markedly.

Another possible explanation for these differences in terms of cooking and cooling is that a higher degree of gelatinization occurred upon boiling varieties 3 and 4 than occurred after boiling varieties 1 and 2. Which is reflected by higher GI values for variety 3 and 4 fresh boiled (Table 6, Figure 2). A higher degree of gelatinization, theoretically should lead to a higher degree of retrogradation when the starch is cooled, resulting in a significant reduction in GI upon cooling as was the case for both variety 3 and 4.

Differences in gelatinization properties between starches can be due to an interplay of a number of previously discussed factors such as, the molecular structure of amylopectin (unit chain length and extent of branching), amylose:amylopectin ratio, and phosphorus content (Liu et al., 2007). Unfortunately, only one of these factors was provided by AAFC; amylose: amylopectin ratio.

As previously indicated the ratio of amylose: amylopectin is one of the main factors affecting starch digestibility and its physiological response (Lehmann & Robin, 2007). Generally, due to its linear structure, amylose is more resistant to hydrolysis by amylase than the highly branched amylopectin molecule. Thus, high amylose starches have been associated with slower rates of or resistance to, digestion (Liu et al., 2007). Based on this, one could assume that the lower GIs after boiling varieties 1 and 2 could be explained by a higher percentage of amylose in the starch, compared to amounts found in varieties 3
and 4. According to the data received from AAFC variety 1 and 2 did contain a higher percentage of amylose (27.1 ±0.2% and 24.3±0.2%, respectively) compared to varieties 3 and 4 (22.7±0.2% and 22.8±0.1%, respectively). The correlation between percent amylose and the GI of fresh boiled potatoes can further support the theory that higher amylose concentrations are associated with lower glycemic responses or GIs. As amylose although not significantly, was inversely related to the GI of fresh boiled potatoes (Figure 4).

In-vitro measurements of RDS, SDS and RS fractions for each variety were correlated with their respected GIs for fresh boiled potatoes as well (Figure 4), which resulted in some unanticipated findings. For example, from the literature (Englyst et al., 1999; Englyst et al., 2003; Englyst et al., 1996a) it was presumed that a higher proportion of RDS would correspond with a higher GI, as high GI is characteristic of a greater glucose load entering the blood relatively quickly. The data indicated however, that RDS was in fact inversely related to the GI of fresh boiled potatoes. As for SDS, we had also anticipated an opposite correlation to what occurred. From the literature (Englyst et al., 1999; Englyst et al., 2003; Englyst et al., 1996a) a higher degree of SDS is typically correlated with a lower glycemic response or GI, as there would be a slower rate of glucose absorption into the blood. The data indicated however that SDS was directly related to the GI of fresh boiled potatoes. Unlike RDS and SDS, RS was not related to the GI of fresh boiled potatoes. Theoretically, the higher the GI, the lower the amount of RS in a food, this non-correlation however is not surprising as the values for RS may be incorrect. Published RS values from cooked potatoes are usually around 1-7 % of TS (Englyst et al.,1992a; Kingman & Englyst, 1994), where the data we received ranged
between 22.5-25.7% of TS from all four varieties. RS is usually calculated (RS=TS-(RDS+SDS)) and not measured, but TS was not provided to us by AAFC, thus it was suggested to use 100 in place of the TS value. The use of 100 instead of the actual percentage of TS, may account for some of the increase in RS values, but not all as the TS values in the literature for potatoes are typically not substantially less than 100 (typically~75-80% of potato dry matter) (Englyst et al., 1992a; García-Alonso & Goñi, 2000; Kingman & Englyst, 1994). This and the unanticipated correlations with RDS and SDS suggest that the in vitro analysis of starch fractions should be repeated, preferably with a greater number of potato samples.

Each starch fraction (RDS, SDS and RS) and amylose was correlated with the GIs for the cold treatments (Figure 5). It should be noted that the in-vitro measurements discussed here are the same values used to correlate with the GI of fresh boiled potatoes. Thus, the measurements were taken from cooked potato starch, so the validity of the correlations is questionable. Nevertheless, unlike the correlations with fresh boiled potatoes, the GI of the cold potatoes was associated with both RDS and SDS as anticipated. The lower the proportion of RDS, the lower the GI of the cold potatoes and the greater the proportion of SDS, the lower the GI, which corresponds with the literature that states low GI is characterized by SDS (Englyst et al., 1999; Englyst et al., 2003; Englyst et al., 1996a). Similarly to fresh boiled potatoes, RS was also not related to the GI of cold potatoes. Even though when cooked potatoes are cooled RS starch has been known to increase to as high as 13% TS (Englyst, et al.1992a) the values obtained from AAFC (22.5-25.7%) are still quite high to be used in the correlation. Amylose although not significantly, is directly correlated to the GI of cold potatoes, which does not correspond with the
literature. Amylose due to its linear structure is able to retrograde and re-form stronger bonds between other starch molecules than the highly branched amylopectin polysaccharide (Englyst et al., 1992a). Retrogradation creates SDS and RS therefore should theoretically result in a reduced glycemic response or GI.

Finally, all varieties responded similarly to being reheated in the microwave after cooling (Figure 2). Generally, the high GI across all four varieties may be explained by the fact that amylopectin (which constitutes the majority of the starch in each variety despite the varying degrees of amylose) when retrograded can be re-gelatinized at lower (70 ºC) temperatures than amylose (160 ºC) (Miles et al., 1985; Ring et al., 1988).

Specifically, in varieties 1 and 2 the GI was higher after the potatoes were reheated then after they were initially boiled. What could have occurred, is that a second round of heating caused the retrograded starch to re-gelatinize and the starch that had initially or was partially gelatinized after boiling to become gelatinized resulting in the higher GI for reheated potatoes. For varieties 3 and 4 it appeared that re-heating caused the retrograded starch to re-gelatinize to the same extent as after fresh boiled potatoes, as the GIs for reheated and boiled potatoes were similarly high. These results are comparable to in-vitro studies investigating the effects of temperature cycling on starch digestibility, as a Marfona potato cooled and then reheated had similar amounts of RDS than when the same variety was freshly boiled and mashed (Kingman & Englyst, 1994).

To investigate further, the different effects of cooking and cooling, which were documented between growing locations, we decided to re-test varieties 2 and 3 the following year. These results are discussed in the following section.
6.2 Study 2

Varieties 2 and 3 tested in study 2 were harvested in the fall of 2009, 1 year later than the potatoes tested in study 1. From four potato meals (variety 2 and 3, fresh boiled and cold), only variety 3 fresh boiled was measured as high GI (72.5), while the other three including variety 2 fresh boiled were all measured as low GI (Table 7). Similarly, to study 1 the GI of variety 3 was significantly reduced when cooled, while in variety 2 there were no significant difference between fresh boiled and cold treatments (F=6.8, p=0.018) (Table 7, Figure 7 and 8 B). The differences between varieties in terms of their response to cooling, supports the theory described in study 1 in that a higher degree of gelatinization occurred after variety 3 was boiled resulting in not only a higher GI but also, a larger proportion of gelatinized starch available for re-crystallization, or retrogradation. This resulted in the significant reduction in GI upon cooling variety 3. Whereas for variety 2, boiling may have not caused a significant disruption to granular structure, resulting in a lower GI and a lesser amount of gelatinized starch available for retrogradation. Although physicochemical data was not provided for the varieties during this study, the results suggest that variety 2, like the variety 2 potatoes tested in study 1, had a higher degree of amylose than variety 3. Other factors such as a greater extent of amylopectin branching (i.e. greater number of long chains) (Zhang et al., 2008), or a lower phosphate content (due to slightly lower proportions of amylopectin) (Karim et al., 2007) may also have contributed to a lesser degree of gelatinization and a lower GI in comparison to variety 3.

Although the response to cooking and cooling were the same for each variety two years in a row (i.e. no year of harvest × treatment interaction (F=1.87, p=0.176)), there was a
significant year of harvest × variety interaction (F=5.1, p=0.027). Specifically, the GI for variety 3 measured during study 2 was virtually the same as its GI measured a year prior (60.5 vs. 59.7, respectively (averaging across fresh boiled and cold treatments). While the GI for variety 2 measured in study 2 (39.2) was significantly less than the GI measured a year prior in study 1 (58.1) (Figure 7 and 8 A). A difference in growing location could not explain the difference in GI between years as variety 2 potatoes were grown in the same location; Vauxhall, Alberta. It was discovered after study completion however, that the potatoes received for study 2 (harvested 2009) were harvested early, at 100 days, compared to the potatoes tested in study 1 which were harvested to maturity (120 days). Potatoes with shorter growing seasons are known to consumers as “new” potatoes and are distinguished by their small shape and tender mouth feel (Camire et al., 2009). The potatoes from study 2 however, looked no different from the study 1 variety 2 potatoes, as both were quite large. Despite this, there has been evidence to suggest that fluctuations in harvest dates can affect starch digestibility.

Soh & Brand-Miller (1999) explained that as potatoes mature, the degree of amylopectin branching significantly increases, which could potentially affect the glycemic response. The quantity of amylose increases only slightly or remains consistent as potatoes mature and thus is not likely to affect the glycemic response. We therefore speculate that the lower GI of variety 2 from study 2 may be due to reduced amylopectin branching resulting in greater resistance to gelatinization. The same study reported the GIs of both boiled new potatoes and canned new potatoes to have lower GIs than the other more mature potatoes tested (Soh & Brand-Miller, 1999). Other than the GI values from Soh & Brand-Miller (1999), there have only been two other values cited for “new” potatoes in
the most recent *International Tables of Glycemic Index and Glycemic Load Values* however, and they are classified as high GIs, with values of 70 and 80 (Atkinson et al., 2008).

There has also been supporting evidence from in-vitro studies to support reduced amylopectin branching in “new” or less mature potatoes and the theory that this may cause some resistance to gelatinization. For example Liu et al. (2003) conducted a study to characterize the physicochemical properties of starches from potatoes of different varieties and growth times, concluding that variation in growth time effects starch properties, and appears to influence varieties differently. Although this study did not investigate amylopectin branching specifically, they did note that the percentage of amylose in the starch was higher for potatoes harvested at the shortest growth time, decreased after first harvest and remained unchanged during the remaining growth period. The decrease in amylose is likely due to an increase in the proportion of amylopectin. This can also be supported by the fact that phosphorus, which is covalently bound to amylopectin, also increases with growth time (Liu et al., 2003). In terms of gelatinization properties, Liu et al. (2003) determined that potatoes with shorter growth time were more resistant to gelatinization than those potatoes with later harvest dates, as indicated by higher gelatinization temperatures and other measurements such starch swelling capabilities and viscosity. This study and other in-vitro studies (Madsen & Christensen, 1996) support the fact that the differences noted between study 1 and study 2 in terms of the GI for variety 2 could be due to differences in physicochemical properties as affected by difference in harvest dates.
Lastly, it is interesting that the differences in growing locations (Fredericton, New Brunswick vs. Guelph, Ontario) for variety 3 between studies did not appear to influence the GI (Figure 8A). This supports the theory, that effect of growing or environmental conditions on starch properties can be dependent on potato variety (Cottrell et al., 1995; Haase & Plate, 1996; Liu et al. 2007).

In summary study 2 has validated the fact that cooking and cooling can affect these specific potato varieties (2 and 3) differently, regardless of harvest year. We were also able to reproduce the same GI values two years in a row for variety 3, but variety 2 differed rather significantly between studies. The latter discovery has prompted us to consider growth times as another determinant of the GI for potatoes. For our third and final study, we wanted to determine if the reduction in GI upon cooling previously cooked potatoes, noted in both study 1 and 2 could be better explained by the malabsorption or slowed absorption of starch. The results of this in-vivo study of starch digestibility are discussed in the following section.

6.3 Study 3

As indicated in the methods chapter, study 3 was conducted during the same harvest year as study 2, and one year after study 1. Therefore similar to study 2, the potatoes tested in study 3 were harvested in 2009. We initially intended to test variety 2 and 3 again; however, variety 1 was used in place of variety 2, as there were not enough variety 2 potatoes left over from study 2.

In terms of GI classifications, both varieties 1 and 3 fresh boiled potatoes were measured as high GI (82.2 and 74.2, respectively), and both variety 1 and 3 cold potatoes were
classified as low GI (48.3 and 50.3, respectively) (Table 8). The GI of variety 1 (82.2) had the highest GI of all potato meals tested over the course of all three studies, interesting as its GI in study 1 was the lowest (59.8) of all four fresh boiled potato meals (Table 6). This difference in GI within the same variety may be due to variation in growing location and conditions, as variety 1 potatoes received for testing during study 3, were harvested in 2009 from Fredericton, New Brunswick, while the potatoes from study 1 were grown and harvested in 2008 from Vauxhall, Alberta. The potential effects of growing locations and conditions on the physicochemical properties of starch were previously discussed in section 6.1. As discussed in section 6.2 there is evidence to suggest that the variation in growing conditions may affect some potato varieties more others than (Cottrell et al., 1995; Haase & Plate, 1996; Liu et al., 2007). This is supported again by the fact that the GI of variety 3 has remained consistent over all three studies, regardless of growing location (Table 6, 7, and 8). As variety 3 in study 1 was grown in Fredericton, New Brunswick (2008), study 2 in Guelph, Ontario (2009), and study 3 in Fredericton, New Brunswick (2009).

With regards to the effects cooking and cooling on the GI of potatoes, unlike studies 1 and 2 there was no interaction between treatment and variety (F=0.792, p=0.385), and only a main effect of treatment (F=26.4, p<0.001) as the GI for both variety 1 and 3 fresh boiled were reduced significantly when cooled (Table 8). This was an interesting observation, as in study 1; there was no difference in GI between variety 1 fresh boiled and cold potato meals. This may be due to the fact that both variety 1 and 3 from study 3, had a high degree of gelatinization during boiling, resulting in a greater percentage of retrograded starch upon cooling leading to a reduced GI. From these differences between
studies in terms of the overall GI for variety 1 and the effects of cooling, it was expected that upon factoring in the GI values from study 1 into the statistical model that there would be a significant year of harvest × variety × treatment (F=5.3, p=0.025) interaction. This represents the fact that the effects of treatment (fresh boiled and cooling) on potato varieties can depend on year of harvest or in the case of study 3 growing location (Figure 10). These differences discussed in terms of GI may be due to difference in starch properties between varieties and even within varieties of different years. Unfortunately, like study 2 physicochemical data was not obtained for the potatoes tested in study 3, and it is difficult to make inferences as it is likely a combination of factors contributing to these differences.

In addition to measuring the glycemic response after the potato meals, to determine their GIs this study also fed a second meal (served 4 hrs after the potato meals), and measured the glycemic response two hours postprandial. A lunch meal was served in study 3 to investigate what is known as the second meal effect (SME). Studies have shown that the glycemic impact of an earlier meal can affect the glycemic response elicited by the next meal. More specifically, that slowed absorption of carbohydrates at an earlier meal has the potential to reduce the glucose response or improve second-meal carbohydrate tolerance (Jenkins et al., 1982; Wolever et al., 1995; Jenkins et al., 1980). Thus, we used the SME theory to investigate whether the formation of SDS or slowed absorption could explain the reduction in GI upon cooling variety 3. We initially thought there would be a lower glycemic response elicited by the lunch meal after consumption of variety 3 cold at the morning test meal versus after consumption of variety 1 cold, as the GI of variety 1 in study 1 was not reduced upon cooling. As recently discussed however, variety 1 in study
3 was affected by cooling, and its GI did significantly lower when cooled. Therefore, after GI data from study 3 was analyzed we expected that there would be no difference in terms of glucose response to a lunch meal between the two varieties. However, we did anticipate that generally the glucose response of the cold treatments (variety 1 and 3 cold) would elicit a lower glucose response to the lunch meal compared to the fresh boiled treatments (variety 1 and 3 fresh boiled), secondary to the formation of SDS after cooling. Results did in fact indicate that there was a main effect of treatment (F=5.6, p=0.029), but interestingly the exact opposite as to what was anticipated occurred as the fresh boiled potatoes caused a significantly lower glucose concentration after lunch than the cold potatoes (5.52±0.13 vs. 5.71±0.11, respectively) (Figure 9). If you consider the glucose profiles, immediately after consumption of the potato meals (0-120min), these differences in glucose concentrations between treatments (fresh boiled vs. cold) may explain why a lower glycemic response elicited at lunch was noted after consumption of the fresh boiled meals rather than after the cold meals. For instance, a substantial rise in blood glucose concentration was noted 30, 60, and 90 min after consumption of fresh boiled potatoes. These concentrations were significantly greater than the concentrations at the same time points after the cold potatoes were consumed (Figure 9). After 90 min the glucose concentrations for fresh boiled potatoes fell quickly, resulting in lower glucose concentrations at 240 min (before the start of lunch) and at 255min (15 min after the start of lunch). This is compared to the cold potatoes, which had a much more gradual fall in blood glucose resulting in significantly higher blood glucose concentrations at 240 and 255 min. The blood glucose profiles after cold potatoes are characteristic of SDS behavior (Lehmann & Robin, 2007), as they appeared to slowly increase postprandial
blood glucose levels (0-120min), and then sustain concentrations over a longer time period (240-360min). This suggests that cooling does create SDS or cause slowed absorption, which may explain much of the reduction in GI between the fresh boiled and cold treatments.

In terms of blood glucose AUC for the standard lunch (240-360 min), there was no main effect of treatment (F=0.52, p=0.482) or variety (F=0.19, p=0.668) and no interaction (F=0.001, p=0.970) was found (Table 8). In other words, the blood glucose AUC after fresh boiled and cold potato meals (0-120 min) had no effect on the glucose response elicited by the lunch meal. Similarly, with the GI values discussed above, there was a main effect of treatment for mean blood glucose AUC after the morning test meals (0-120 min) (F=20.4, p<0.001). Cold potato meals had a significantly lower AUC than the AUC for the fresh boiled potatoes (122±15 vs. 203±23, respectively) (Table 8). The reduction in blood glucose profiles (0-120 min), GI and AUCs after cooling fresh boiled potatoes suggest formation of SDS or insinuate that slowed absorption has occurred, even though an improved second meal glucose tolerance was not indicated (no SME).

We may not have seen a SME in the current study for one or a combination of three reasons. First, the amount of SDS formed upon cooling may not have been enough to affect the glucose response of a later meal. For example in many of the studies that have documented improved glycemic response to a later meal, the earlier meals contained foods such as whole barley, guar and lentils as their slowly digestible components (Jenkins et al., 1982; Jenkins et al., 1980; Nilsson, 2008; Wolever et al., 1988 Priebe et al., 2010). Potatoes upon cooling are shown to contain approximately 2.1 g of SDS and 1.1 g of RS in a 100g edible portion (Englyst & Hudson, 1996a; Kingman & Englyst,
While from the same study (Englyst & Hudson, 1996a), barley (7.0g of SDS and 2.1g of RS in a 100g edible portion) and lentils (6.1 SDS and 2.4 RS 100g edible portion) have been shown to contain greater amounts of SDS and slightly higher amounts of RS. Guar is a viscous fibre and is not absorbed by the small intestine, and like other fibers is known for its effects on slowing the rate of absorption of other available carbohydrates. Therefore the proportion of SDS, RS and fibre included in the earlier meals of the studies listed above are likely able to slow the rate of absorption better than SDS and RS from a cold potato resulting in a SME. Second, the differences in GI between fresh boiled and cold treatments may not have been significant enough to cause differences between the glucose responses after the lunch meal. Some of the studies reporting SMEs have investigated foods or meals that have larger differences between their GIs than the difference noted between the fresh boiled and cold GIs for the current study (78.2±7.9 vs. 49.3±5.7, respectively, values averaged across variety) (Table 8). For example a study by Wolever et al. (1988) reported a low GI meal (GI=40) to significantly reduce both the acute postprandial blood glucose response to dinner and the glycemic response to a subsequent breakfast compared to that of a high GI meal (GI=105). The difference between the GIs in the Wolever et al. (1988) study was 61%, compared to a 36% difference between the GIs of the fresh boiled and cold potato meals in study 3. Lastly, the other possible reason a SME was not documented could be due to the composition of the standard lunch meal. For example, the standard lunch served to subjects in the current study was more or less a complete meal providing 17% of calories from protein, 29% from fat and 54% of calories from carbohydrate with 2.1g of fibre. Some of the second meals from other studies included simpler meals such as solutions of glucose (Wolever et
al., 1988; Jenkins et al., 1980; Priebe et al. 2010), cornflakes with 2% milk (Wolever et al., 1988) and white bread with water (Nilsson et al., 2008). There have been other studies however, that have documented SMEs, that fed more substantial meals similar to the current study (Jenkins et al., 1982). Perhaps the effects of slowed absorption on a second meal is more pronounced or better documented if the later meal contains a simple or more rapidly absorbable food component such as glucose. Instead of a more complete meal, including fat, protein, fibre and more complex carbohydrates which could minimize the effects of SDS from the earlier meal.

To summarize the blood glucose response elicited by a lunch meal was not reduced after consumption of cold potato meals compared to when fresh boiled potatoes were consumed. This non-reduction is likely due to the factors mentioned above rather than to a greater proportion of RS than SDS creation upon cooling fresh boiled potatoes (discussed below).

Lactulose has been widely used as a standard in breath hydrogen measurement. As previously described, breath hydrogen can be a noninvasive way of detecting carbohydrate malabsorption in humans. Lactulose is commonly used to represent a known amount of malabsorbed carbohydrate in order to obtain a semi-quantitative estimate of the amount of unabsorbed carbohydrate in a test meal (as described in the methods chapter) (Wolever, 2006; Owira et al., 2005). The use of lactulose to generate hydrogen and the ability of the Quintron system to collect and measure it in breath was validated by the fact that hydrogen concentrations for lactulose were significantly greater than after the potato meals, in six out of the nine concentration points (P<0.05) (Figure 11). The total hydrogen produced after lactulose (233.4±34.9 ppm), was also significantly
greater than all four potato meals (28.6 ± 6.7 ppm to 81.0 ± 31.1 ppm) and white bread (19.3 ± 5.0 ppm) (P < 0.05) (Table 9).

As anticipated, cold potatoes generated significantly more total hydrogen than fresh boiled potatoes (61.2 ± 19.4 ppm and 33.9 ± 9.4 ppm, respectively) (F = 6.4, p = 0.021) (Table 9). This corresponded with the estimation for malabsorbed carbohydrate in each of the potato meals, as cold potatoes had significantly greater malabsorbed carbohydrate or RS than the fresh boiled potatoes (2.5 ± 0.4 g and 1.6 ± 0.3 g, respectively) (F = 6.4, p = 0.021) (Table 10). It is also interesting to point out that variety 1 and 3 cold potatoes had significantly greater total breath hydrogen and contained more malabsorbed carbohydrate than white bread (P < 0.05) (Table 9 and 10).

For the purpose of comparison, the average amount of RS found in variety 1 and 3 cold was 0.9 g in 100g edible portion, and the amount found in variety 1 and 3 fresh boiled was 0.6 g. These are comparable to the RS values from Kingman et al. (1994), which determined the starch fractions of different potato varieties cooked and cooled, using the Engyst et al. (1992a) method. For example a Marfona boiled, fresh cooked potato contained 0.3 g of RS, while the same variety cold contained 1.2 g of RS (per 100 g edible portion). A boiled, hot, Belle Fontenay potato from the same study had a RS content of 0.2 g, which increased to 0.8 g when cooled (per 100 g edible portion) (Kingman et al. 1994). The RS measurements for the hot potatoes from the literature tend to be lower than the amount of RS measured in the current study for fresh boiled potatoes. It is also interesting that the average difference in RS between the hot and cold potatoes for the values cited is 0.8 g/100g, while the average difference between the hot and cold potatoes in study 3 is only 0.3 g/100g. This suggests and supports the discussion
in section 6.1 in that the novel potatoes tested have potentially been bred as hoped, in that they contain a slightly higher proportion of RS than other commercial varieties.

One of the main goals of study 3; however was to determine if the significant reduction in GI upon cooling some of the novel potato varieties could be explained by an increase in RS content. We conclude that this is not likely the case, as the reduction in GI or blood glucose is much larger than can be accounted for by the reduction in the amount of digestible starch. To use study 3 as an example, the cooked potato starch from variety 1 and 3 contained an average of 1.6 g of RS per serving (~268 g) which increased to 2.5 g upon cooling (Table 10). Thus the 50g “available” carbohydrate portion of cold potatoes would therefore contain 47.5 g of digestible starch, compared to 48.5 g in the fresh boiled potatoes. This 2.1% reduction in digestible starch cannot possibly account for the 37% reduction in GI noted in study 3 when fresh boiled potatoes were cooled. Similar rational is discussed in Fernandes et al. (2005). So although there is an increase in RS upon cooling these novel potatoes, it is likely that the reduction in GI is better explained by the creation of SDS or slowed digestion, even though a SME was not found.

Potential limitations to the breath hydrogen method for a means of estimating malabsorbed carbohydrate should be discussed. A more detailed discussion however, is outlined in Chapter 1, as the limitations discussed will only include those relevant to the interpretation of the results from study 3. One of the main criticisms of the breath H₂ method is the perception that it has the inability to detect small changes in breath H₂ output (Robb et al., 1991) or is not sensitive enough to detect differences in malabsorbed carbohydrate of less than 10g (Grysman et al., 2008). This was not indicated in the current study, as small differences much less than 10 g of RS were identified. However,
perhaps a more appropriate method for estimating RS could be to determine the differences between individual potato meals for both breath hydrogen and malabsorbed carbohydrate values, as the only significant differences noted in this study were between the main effects of treatment (fresh boiled vs. cold).

The fermentable capabilities of fibre have also been thought of as a contributor to hydrogen exhalation (Scheppach et al., 1991). However a study by Robb et al. (1991) showed that the addition of 15 g of the fermentable fibre guar gum, did not contribute to any significant production in H₂ over 12 hrs. The amount of fibre in variety 1 and 3 test meals contained much less than 15g as variety 1 contained 3.5 g of fibre in a 235 g serving and variety 3 contained 5.4 g of fibre in a 301 g serving.

Finally the use of both lactulose and inulin have been used as the standard in breath hydrogen measurement, and there is ongoing debate as to which is more valid (Owira et al., 2005). A study looking at the effects of using either inulin or lactulose as the standard in controls and patients with chronic pancreatic exocrine insufficiency indicated that maximum hydrogen production was significantly greater in both groups, with lactulose than with inulin (Owira et al., 2005). This suggests that the colonic bacteria metabolism of lactulose produces more hydrogen than that of inulin. This study also stresses that due to these differences, breath hydrogen studies that have used different standards (i.e. inulin vs. lactulose) should not be used to compare hydrogen concentrations or amounts of malabsorbed carbohydrate between foods (Owira et al., 2005).

These and other limitations of this method should be considered when measuring and interpreting breath hydrogen. However, based on the supporting literature we feel breath
hydrogen is a useful and fairly accurate method for investigating carbohydrate malabsorption in-vivo.
Chapter 7

Conclusions and Implications

The results of study 1 and 2 supported our hypothesis, in that the effects of preparation method, specifically cooling on the GI was dependent upon the variety of potato. This was confirmed two harvest years in a row. We were also able to confirm the GI of variety 3, as it was virtually the same in both studies 1 and 2. We were unable to confirm however, the GI of variety 2, as its GI differed significantly between study years. This variation was hypothesized to be due to differences in growing times between studies for that particular variety.

The GI of variety 3 was confirmed as well as its response to cooling for a third and final time in study 3. Interestingly, cooling variety 1 significantly reduced its GI; while in study 1 cooling had no effect on this variety. Thus, study 3 identified a year of harvest ×variety ×treatment interaction, meaning that the effect of treatment (fresh boiled and cold) was dependent on the variety of potato and the year it was harvested. The difference in terms of the effects of cooling across harvest years for variety 1 may be due to the fact that potatoes from study 1 were not grown in the same place as study 3.

After evaluation and estimation of slowed absorption and malabsorption of potato starch, we were able to support our hypothesis in that, the increase in RS upon cooling a fresh boiled potato was not nearly large enough to explain the 37% reduction in GI. We speculate that the majority of the reduction was likely due to a reduction in the rate (i.e. creation of SDS) rather than the amount of starch absorbed.
All three studies have re-emphasized the importance of factors such as preparation method, harvest dates and growing conditions to the glycemic response of potato starch. Unfortunately, limited physicochemical data was received, and we were unable to investigate how these different factors can interplay to affect the properties of starch, ultimately leading to differences in potato starch digestibility. However, from GI testing alone, we were able to identify low GI or lower GI potatoes and perhaps provided a different perspective to measuring and interpreting the GIs of potatoes.

Potatoes in our minds remain a healthy food choice. If the ultimate goal however, is to increase consumption of low GI or slowly digested foods, potatoes can be considered, as not all are created equal.
Chapter 8

Future Directions

In the development of all three studies and through the interpretation of their respected results, we have indentified some opportunities for future work around the variables effecting potato starch digestibility. First, a greater number of novel varieties should be subjected to GI testing, to determine the effects of cooking and cooling, and whether these effects differ between varieties. It would also be interesting to re-investigate starch digestibility in-vivo with a greater number of varieties through measurement of breath hydrogen and the glycemic response of a lunch meal and see if a larger sample of potatoes may identify greater differences.

Potato maturity and the variation in growing conditions were identified as potential factors affecting the glycemic response. It would therefore be interesting to conduct another study to investigate different growing times to determine the effects on different physicochemical properties, and how this can influence the GI of different varieties.

Lastly, and perhaps most importantly, physicochemical data is required to better interpret the in-vivo data. Better collaboration would be helpful with plant breeders who provide the novel potato varieties for testing, to better understand the genotype of each variety and how they differ from other varieties received. Any future work should also involve the starch chemists as well to link and explain what is occurring physiologically, with the properties of the potato starch.
References


responses to test meals in subjects with noninsulin-dependent diabetes mellitus. 


