The Effects of *Salvia hispanica L.* (Salba) on Postprandial Glycemia and Subjective Appetite

by

Amy Sanda Lee

A thesis submitted in conformity with the requirements
for the degree of Master's of Science

Nutritional Sciences
University of Toronto

The Effects of *Salvia hispanica L.* (Salba) on Postprandial Glycemia and Subjective Appetite

Amy Sanda Lee

Master's of Science

Nutritional Sciences
University of Toronto

2009

Abstract

Dietary interventions have been attempted to lower the risk of obesity, diabetes and CVD by the reduction of postprandial hyperglycemia and prevention of excess caloric intake. Evidence suggests an independent predictive role of postprandial glycemia for CVD. Furthermore, due to the possible role of obesity in the development of CVD and T2D, research has focused on appetite suppression to reduce excessive food intake. Here we investigate the ability of the novel oil-rich grain *Salvia hispanica L.* (Salba) to lower postprandial glycemia and reduce appetite when added to a carbohydrate meal. In our first study, we investigated the effects of Salba in escalating doses on both parameters in healthy individuals. In our second study we compared the effectiveness of ground and whole forms of Salba on the same parameters. Results confirmed our hypotheses, as Salba given in either form positively affected postprandial glycemia and mildly suppressed appetite.

Acknowledgments

I thank Dr. Vladimir Vuksan for taking a chance with a student with absolutely no nutrition background or research experience whatsoever, and Elena, Pearl, Leanne, Andre, Agnes and Alex for their neverending support and expertise. I would also still be analyzing my data if it weren't for the help of the wonderful volunteers at the Risk Factor Modification Centre: Zazeba, Hans, Cathy, Carmen and Nirangive. I thank my advisory committee members Dr. Valerie Tarasuk and Dr. Thomas Wolever for their guidance and for always keeping my best interests in mind. Lastly, I thank my parents, Win and Stephen Lee, for being such willing participants and for their emotional and financial support, and Dennis, for always being there.

Table of Contents

List of Tables

List of Figures

List of Appendices

1 Introduction

Today there is much public interest in improving overall health through diet, with increased interest in whole grains and seeds such as wheat, oats and flax. *Salvia hispanica L.* (Salba) is an ancient oil-rich whole grain that has a favourable nutritional composition compared to commonly consumed grains and seeds. Traditionally considered a food and remedy by ancient Aztec and Mayan civilizations, it is no longer consumed except for in limited areas of Mexico [1]. Awareness of the benefits of functional foods has spurred the search for less frequently consumed foods to complement the North American diet [2, 3]. Salba may prove to be an excellent functional food given its unique and superior composition.

Several preliminary studies indicate potentially beneficial physiological effects of Salba on risk factors for Type 2 diabetes and CVD. In a 6-month crossover study by Vuksan *et al.*, Type 2 diabetic subjects consuming 37g of Salba per day experienced lower blood pressure, low-grade body inflammation and coagulation factors compared to wheat bran control [4]. Vertommen *et al.* reported a significant reduction in waist circumference in healthy individuals after a month of Salba supplementation. This decrease occurred without a change in body weight, suggesting the specific loss of fat mass. Similarly, dyslipidemic rats fed Salba for 3 months experienced lower visceral adiposity than rats on a control maize diet [5]. We hypothesized that the mechanisms of action responsible for the effects seen in these three studies were mainly the reduction of both postprandial glycemia and appetite.

We investigated the effects of Salba on postprandial glycemia and subjective appetite, both of which have been suggested to play a role in of the development of Type 2 diabetes and CVD. The research objectives of this thesis were to assess the ability of Salba to lower postprandial glycemia and appetite in escalating doses in ground form (Study 1) and whole versus ground form (Study 2) using a crossover, randomized, double-blind placebo-controlled design. Results from these studies may provide possible explanations for the long-term effects of Salba seen in previous research in Type 2 diabetes and healthy individuals. If Salba proves to provide health benefits as a functional food, its use can be further developed to complement the Western diet and provide consumers with a novel grain to help them adhere to dietary whole grain recommendations.

2 Review of Literature

2.1 Introduction to *Salvia hispanica L.* (Salba)

2.1.1 Background and Classification

Salvia hispanica L., commonly known as Chia, is an oil-rich grain that has been consumed for thousands of years [1]. It was one of the Aztec and Mayan cultures' three main crops, along with amaranth and corn. Called "running food", it was revered for its medicinal properties and exceptional nutritional value; so much so, that it played a prominent role in certain religious ceremonies [1]. Recently, renewed interest has emerged for this grain, as researchers have uncovered its extraordinary composition and potential beneficial effects on health.

Chia is the mother crop of *Salvia hispanica L.*, and consists of many varieties with varying nutritional compositions. Selective breeding of this mother crop yielded a white grain with a more consistent nutrient composition and greater nutrient density [6]. We studied this variety of *Salvia hispanica L.*, known commercially as "Salba," which is rich in dietary fiber, omega-3 fatty acids, minerals and vegetable protein, and which has a high antioxidant capacity as well.

Although traditionally considered a grain, it can also be regarded as a seed due to its high oil content. As well, it does not belong to the *Graminaea* family, in which all major cereal grains are conventionally classified. Excluding its low carbohydrate content, however, Salba contains dietary fiber, unsaturated fatty acids and other phytochemicals which render it compositionally similar to typical grains. Thus, Salba may prove to have the health benefits of more commonly consumed whole grains seen in epidemiological and interventional studies [7-14]. Many of the beneficial effects of whole grains are attributed to their contents of such nutrients as dietary fiber, minerals, unsaturated fatty acids, vegetable protein and antioxidants [10, 15].

Exceptionally high in fiber, Salba is also rich in minerals such as iron, calcium, magnesium and potassium [16, 17] and has an exceptional total antioxidant capacity (TAC) of 84/g, making it comparable to some berries [18]. With Salba's fat content of 33%, it contains considerably more fat than the typical whole grain. As such, is also similar to seeds such as flax. Currently however, flax is considered a whole grain [19]. Salba may also be called a whole grain since it is considered as one by the medical community [4, 20].

2.1.2 Composition of Salba (Please refer to Tables 2.1 and 2.2)

Table 2.1 Macronutrient Composition of Salba and Common Grains (100g)

	Whole Grain Wheat	Oats	Flax	Salba
Calories	**339 kcal**	**389 kcal**	**534 kcal**	**389 kcal**
Total Fat	**2.0 g**	**6.9 g**	**42.0 g**	**33.0 g**
Saturated	0.3 g	1.0 g	3.7 g	2.4 g
Polyunsaturated	0.8 g	2.5 g	28.7 g	28.7 g
Omega-3	0.038 g	0.1 g	22.8 g	22.0 g
Omega-6	0.7 g	2.4 g	5.9 g	6.3 g
Monounsaturated	0.2 g	2.2 g	7.5 g	1.8 g
Total Carbohydrate	**73.0 g**	**66.3 g**	**29.0 g**	**35.0 g**
Dietary fiber	12.0 g	10.6 g	27.0 g	34.0 g
Sugars	0.4 g	0.0g	1.5 g	0.0 g
Available CHO	61.0 g	0.0g	2.0 g	1.0 g
Protein	**14.0 g**	**17 g**	**18.0 g**	**22.0 g**

From the USDA Nutrient Data Laboratory [21] *and Nutrition Data* [22] *; Salba nutrient information determined by laboratory nutrient analysis at the University of Guelph and University of Toronto* [17, 23-25]

Table 2.2 Micronutrient Composition of Salba and Common Grains (100g)

	Whole Grain Wheat	Oats	Flax	Salba
Thiamin	0.4 mg	0.76 mg	1.64 mg	0.74 mg
Riboflavin	0.2 mg	0.14 mg	0.161 mg	0.20 mg
Niacin	6.4 mg	0.96 mg	3.08 mg	7.15 mg
Vitamin B6	0.3 mg	0.12 mg	0.47 mg	0.10 mg
Folate	0.04 mg	0.06 mg	0.0 mg	0.08 mg
Vitamin C (Ascorbic Acid equivalency)	0.0 mg	0.0 mg	0.6 mg	5.4 mg
Calcium	34.0 mg	54.0 mg	255.0 mg	770.0 mg
Iron	3.9 mg	4.7 mg	5.73 mg	7.9 mg
Magnesium	3.8 mg	177 mg	392.0 mg	380.0 mg
Phosphorus	346.0 mg	523 mg	642.0 mg	780.0 mg
Potassium	405.0 mg	429 mg	813.0 mg	660.0 mg
Zinc	2.9 mg	4.0 mg	4.34 mg	4.4 mg

From the USDA Nutrient Data Laboratory [21]; Salba nutrient information determined by laboratory nutrient analysis at the University of Guelph and University of Toronto [17, 24, 25]

2.1.2.1 Carbohydrates and Dietary Fiber

Salba is composed of 35% total dietary carbohydrate, of which 34% is in the form of dietary fiber [25]. Thus, merely 1% of Salba is available carbohydrate. Dietary fiber can be defined as the "edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated plant substances." [26] For example, the structural supports of plants' stems and leaves are considered dietary fiber. All types of fiber are non-starch polysaccharides except for lignin, which is an alcohol derivative [27].

A common method of classifying fibers is by their solubility in water and ability to gelatinize [28, 29]. Soluble fibers are hygroscopic and able to form a gel, and include psyllium husks, guar, beta-glucan from oats and barley and naturally-occurring pectins in fruit [27]. The soluble fibres are typically fermented to a greater extent than insoluble fibres, although all non-starch polysaccharides are partially fermented to some degree in the large intestine [30].

According to the American Association of Cereal Chemists, dietary fibers promote beneficial physiological effects including laxation, and blood cholesterol and glucose attenuation [26]. Research supports this claim: it has been found that fiber improves glycemic control [31, 32], lowers blood lipid levels [33] and may even reduce the risk of colon cancer [34].

Table 2.3 Total Percentage of Dietary Fiber Content of Certain Common Grains

	Whole Grain Wheat	**Oats**	**Flax**	**Salba**
Soluble fiber	10.2 %	5 %	10 %	2.3 %
Insoluble	2.0 %	6 %	17 %	32 %
Total fiber	12.2%	11 %	27 %	34 %

From the USDA Nutrient Data Laboratory [21]*; Salba nutrient information determined by laboratory nutrient analysis at the University of Guelph and University of Toronto* [17, 23-25]

2.1.2.2 Dietary Fat Content

Salba is composed of approximately 33% fat. Of that, 68% is in the form of omega-3 polyunsaturated fatty acid, 19% omega-6 polyunsaturated fatty acid, 6% monounsaturated fatty acid and 16.4% saturated fatty acid. The omega-3 polyunsaturated fat is in the form of alpha-linolenic acid [24]. Polyunsaturated omega-3 fatty acids include the 18-carbon alpha-linolenic acid (ALA), 20-carbon eicosapentaneoic acid (EPA) and 22-carbon docosahexaneoic acid (DHA). Animal products such as fish are excellent sources of EPA and DHA, while ALA can be found in plant foods such as vegetables and seeds. They are all the precursors to eicosanoids including prostaglandins, thromboxanes, and leukotrienes, which have extensive hormonal functions in the body. Yet they are all essential fatty acids, meaning that the body is unable to produce them. However, the human body has a limited ability to form EPA and DHA from ALA [35-37]. These reactions occur competitively with omega-6 fatty acids, and as such, the formation of long-chain omega-3 fatty acids is most effective when their number is not significantly less than that of the omega-6 fatty acids [38, 39].

Thus the ratio of dietary omega-3 to omega-6 fatty acids is especially important given the competition between omega-3 and omega-6 analogues for the same conversion pathway. The conversion of ALA to EPA is estimated to be anywhere from 0.2 to 21%, and further synthesis of EPA to DHA is especially limited with approximately 0-9% of ALA being metabolized to DHA [40-42]. The efficiency of this pathway varies between species and even between sexes, with women demonstrating a greater capacity to convert EPA and DHA from ALA [37, 41, 42]. Unfortunately, it is estimated that the typical North American consumes omega-6 and omega-3 fatty acids in ratios between 14:1 and 20:1, while scientists recommend a ratio of 4:1[36]. Thus, regular ingestion of Salba may help counteract the imbalance of omega-6 to omega-3 fatty acids in the typical North American diet given its ratio of 1:3. Preliminary data suggest that Salba increases blood levels of EPA. After participants consumed approximately 37g a day for 12 weeks, their ALA and EPA blood levels nearly doubled, indicating the effective conversion of ALA to EPA [4].

Experimental and epidemiological studies have demonstrated that ingestion of fish oil, which is rich in both EPA and DHA, reduces risk factors associated with cardiovascular disease such as hypertension and insulin resistance [37, 40, 43]. Fewer studies have investigated the effects of ALA on such parameters, but preliminary results suggest that this form of polyunsaturated fat may also play a beneficial role. For instance, a 2-year study assessing the intake of ALA-enriched margarine versus LA-rich margarine reported significantly lower c-RP levels in the former diet condition [43]. In addition, perilla oil (rich in ALA) effectively suppressed fatty acid synthase activity and decreased

hepatic and plasma triacylglyerol levels compared to maize oil (rich in linoleic acid) in rats [44]. Further studies in rats suggest that regular ingestion of ALA from Salba can significantly decrease diet-induced dyslipidemia compared to ingestion of linoleic acid (LA) in the form of maize oil [5]. It is important to note, however, that conversion of ALA to EPA is more efficient in rats than in humans. Nonetheless, these preliminary results are promising and warrant further study into the effects of dietary ALA on possible risk factors for CVD.

2.1.2.3 Dietary Protein Content

Salba contains 22% vegetable protein [17]. The quality of a protein, usually expressed as a Protein Efficiency Ratio (PER), is dependant on the percentage of protein that is likely to be used by the body [45]. The PER is measured by feeding rats a diet containing 9-10% of the protein for 4 weeks and calculating the weight gain per unit of protein consumed. The value is then adjusted proportionately to the PER that would be obtained if the PER of a casein diet were 2.5. Casein, derived from skim milk, is a high quality protein and thus a standard of comparison of protein quality [46].

The adjusted PER of Salba is 91% (using casein as the standard). The PER of Salba is higher than that of soy protein, a highly regarded source of protein often used as a meat substitute by vegetarians [16]. Additionally, the protein in Salba has no limiting factors for the adult diet (based on the limiting amino acid, lysine) meaning that it contains all essential amino acids. Thus, it is a complete and balanced source of protein [1]. As well, Salba contains no gluten, a protein found in wheat [1]. Therefore, it can safely be safely consumed by individuals with celiac disease.

Table 2.4 Amino Acid Composition of Salba

Amino Acid	Salba (100g)
Alanine	314 mg
Arginine	518 mg
Aspartic acid	546 mg
Cystine	102 mg
Glutamic Acid	1080 mg
Glycine	298 mg
Histidine	174 mg
Isoleucine	218 mg
Leucine	410 mg
Lysine	288 mg
Methionine	102 mg
Phenylalanine	312 mg
Proline	230 mg
Serine	354 mg
Threonine	294 mg
Tryptophan	666 mg
Tyrosine	142 mg
Valine	310 mg

Nutritional laboratory analysis performed at the University of Guelph [23]

2.1.2.4 Antioxidant Capacity

An antioxidant is a substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly prevents or delays oxidation of the substrate triggered by a pro-oxidant [47]. A pro-oxidant, or reactive oxygen species, is a toxic substance that can cause oxidative damage to lipids, proteins and nucleic acids, resulting in various pathologic events or diseases. The

antioxidant capacity of a compound is defined as the ability of the compound to reduce pro-oxidants [47].

Antioxidant capacity is often expressed as Total Antioxidant Capacity, or TAC, per gram. The TAC is the sum of the oxidant radical antioxidant capacity (ORAC), as determined with an ORAC assay, of both the lipophilic and hydrophilic components of the compound [18]. Salba has a TAC value of 84/g, while that of lowbush blueberries, generally considered an excellent source of antioxidants, is 96/g. Berries such as the raspberry and strawberry have TAC's of 49/g and 36/g, respectively [48].

Water and methanol extracts of the Chia meal remaining after pressing to remove oil demonstrate high antioxidant activity [1]. It is because of this antioxidant activity that the omega-3 fatty acids in Salba are very stable and also why Aztecs were able to store the grain for long periods of time with low risk of rancidity. Scientific interest on the health benefits of antioxidant consumption has focused mainly on the effects of beta-carotene and vitamins E and C on CVD [49]. Numerous epidemiological studies have demonstrated an association between the consumption of antioxidant-rich foods such as fruits and vegetables and nutrients such as vitamin E and flavonoids and lowered risk of mortality from cardiovascular events [49-56]. One 2-year interventional trial called the Cambridge Heart Association Study reported that consumption of vitamin E reduced the risk of CVD-related events such as non-fatal myocardial infarction [57]. Most randomized trials, however, show no protective effects of antioxidant supplementation on cardiovascular events [49, 58-60]. Therefore, although some health benefits have been documented, more research is needed.

Table 2.5 Antioxidant Composition of Salba

Compound	Concentration (mol/kg Salba)
I – Nonhydrolyzed	
Flavonols	-----
Cinnamic acids	-----
Caffeic acid	6.6×10^{-3}
Chlorogenic acid	7.1×10^{-3}
II – Hydrolyzed	
Flavonols	-----
Myricetin	3.1×10^{-3}
Quercetin	0.2×10^{-3}
Kaempferol	1.1×10^{-3}
Cinnamic acids	-----
Caffeic acid	13.5×10^{-3}

From © Salba Group http://www.salba.info/antioxidants.html [61]

2.1.3 Salba: Past Clinical Research

The field of nutrition has recently begun to focus on the benefits of foods beyond fulfilling basic nutrient requirements; such foods are called "functional foods" [3]. Due to a rich nutrient composition and promising preliminary clinical data, Salba is a grain that could be considered a novel functional food. Past studies done with Type 2 diabetics, healthy individuals and rats have all demonstrated a possible role for Salba in improving risk factors for Type 2 diabetes and CVD such as glucose metabolism and adiposity. However, very little research has been conducted with this grain.

In a 6-month randomized, crossover study, Salba demonstrated the ability to decrease risk factors for CVD in Type 2 diabetes [4]. Diabetic individuals undergoing conventional therapy and adhering to a CDA-recommended diet consumed 37g of either Salba or wheat bran (control) per day for 3 months.

The Salba group experienced a significant reduction in systolic blood pressure of 6.3 ±4.2 mmHg compared to baseline [4]. Although it did not reach significance, diastolic blood pressure dropped as well, by an average of 1 ±1.3 mmHg compared to baseline. Likewise, a significant decrease in HbA1c was seen in the Salba group compared to baseline, but not to control. With regards to coagulation factors, fibrinogen and VonWillebrand factor levels in the Salba group both decreased significantly compared to baseline only. Low-grade body inflammation, as measured by c-RP, was significantly lower in the Salba group compared to the control group at the end of the 12 weeks.

Vertommen *et al*. conducted a 1-month fluidity study in which 12 health individuals consumed 50g of Salba per day [20]. Diastolic blood pressure decreased significantly from 66.1 ± 8.4 to 61.5 ± 7.0 mmHg. Fasting serum triglycerides tended to decrease from 89 ± 52 to 69 ± 22 mg/dL ($p = 0.07$). Finally, a decrease in waist circumference was reported without a concurrent decrease in body weight, which could be due to the specific loss of fat mass. No side effects were observed in either study and all safety parameters remain unchanged. Both authors speculated that Salba's beneficial health effects were due to the rich nutrient content naturally occurring in this ancient grain.

Most recently, Chicco *et al.* studied the effects of a diet including Salba in rats on parameters such as insulin sensitivity and visceral adiposity. The animals were placed on a 5-month sucrose-rich diet to induce dyslipidemia and insulin resistance, followed by a diet enriched with either Salba or maize [5]. The objective was to compare the effects of the ALA from Salba versus linoleic acid (LA) from maize. Results demonstrated a significant decrease in visceral adiposity (epididymal and retroperitoneal fat) relative to body weight for the rats on the Salba diet. Thus these results also suggest a role for Salba in lowering adiposity. With regards to insulin sensitivity, it was found that insulin resistance was normalized without changes in insulinaemia in the rats receiving the Salba diet.

The results of these three studies suggest a possible role for Salba in the prevention of risk factors for Type 2 diabetes and CVD, as it has been demonstrated that Salba reduces blood pressure, coagulation factors and adiposity. Given the promising results of these studies, Salba demonstrates potential as an excellent functional food, and as such, further research into this grain may prove to be beneficial for human health.

2.2 Postprandial Glycemia

2.2.1 The definition of Postprandial Glycemia

Postprandial glycemia refers to the elevations of blood glucose concentration that normally occur in response to a meal [62]. In healthy individuals, the ingestion of a typical carbohydrate-rich meal causes transient increases in plasma glucose that peak approximately 30-60 mins after the meal and return to the fasting, preprandial level in approximately 2-3 hours, depending on the type and amount of carbohydrate. Levels of postprandial glycemia in healthy, nondiabetic individuals rarely exceed 7.8-8.8 mmol/L; however, they can peak at 10 mmol/L after some large meals [62, 63]. The dynamics of the postprandial glucose response are tightly regulated as part of the body's glucose homeostasis such that postprandial variations in glycemia reflect the interplay between intestinal absorption, endogenous release and tissue uptake of glucose from circulation.

Glucose is absorbed from the small intestine through the secondary active sodium-glucose cotransporter SGLT-1 and sodium-independent facilitative transporter GLUT-5 situated in the brush-border membrane [64]. SGLT-1 activity is mainly regulated by diet [65]. Studies show however, that angiotensin II may play a role in regulating sodium-glucose cotransport. In animal models, angiotensin II was shown to inhibit SGLT-1 activity by suppressing its translation and to increase SGLT-2 expression and function in tubular cells [66]. From the enterocytes, glucose reaches the liver through the portal vein. Here, high postprandial glucose concentrations activate glucokinase, in turn promoting hepatic glucose uptake. At the hepatic level, glucose exceeding immediate needs is stored as glycogen or converted to fat. The rest of the glucose is transported into the circulation and reaches pancreatic beta cells where it stimulates insulin release and secretion, as glucose is the most powerful stimulator of insulin production and release [67].

Insulin is produced in the beta cells of the Langerhans pancreatic islets. Insulin release is triggered by high postprandial glucose concentrations following a carbohydrate meal, which activates the beta-cell glucokinase with a low affinity for glucose. Consequently, increased amounts of glucose enter the citric acid cycle producing large amounts of ATP, and subsequently insulin exocytosis is stimulated [68].

The endogenous hepatic and renal glucose release and tissue uptake of glucose from circulation are antagonistically regulated by insulin and glucagon, the latter hormone of which is secreted by pancreatic alpha cells [68]. The absorbed glucose stimulates insulin secretion and suppresses glucagon secretion, which together cause an 80% reduction in endogenous glucose production by suppressing

hepatic glycogenolysis and gluconeogenesis [68]. The rate-limiting step for cellular glucose uptake is glucose transport, enhanced by both hyperglycemia and insulin.

Up to 75% of the glucose available postprandially is taken up by an insulin-independent mechanism. Another 25% is taken up through insulin-dependent GLUT-4 transporters in peripheral tissues, mainly in skeletal muscle (80%) and to a lesser extent in heart and adipose tissue [68]. Moreover, in these tissues, insulin directs intracellular glucose metabolism by activating key enzymes such as glycogen synthase and pyruvate dehydrogenase, while suppressing lipolysis, inhibiting hepatic gluconeogenesis, increasing glucose uptake in the muscle and promoting glucose oxidation [69]. Incretin hormones such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide (GIP), which are released distally in the ileum upon carbohydrate intake, amplify insulin secretion beyond the levels induced by glycemic increases [70].

2.2.2 The Clinical significance of Postprandial Glycemia

Postprandial glycemia reflects the body's ability to regulate glucose levels. Based on this principle, an individual's glycemic response to a set amount of carbohydrate can be a diagnostic tool for insulin resistance and the onset of diabetes. There is much interest in reducing postprandial glycemia in people at risk for diabetes since doing so appears to decrease diabetes risk and associated cardiovascular complications [71, 72]. Most importantly, growing epidemiological evidence suggests that postprandial glycemic rises are an independent and modifiable predictor of cardiovascular disease. Studies show that, even after adjusting for other cardiovascular risk factors, the relationship between 2-hour postprandial glycemia and cardiovascular risk remains direct and continuous, extending below and beyond the cut-off points for impaired glucose tolerance [737475]]]. Furthermore, postprandial glycemic spikes have detrimental effects on blood coagulation, body inflammation and endothelial cell function and, together with 2-hour glycemia, are considered equally predictive of atherosclerosis [33-36].

Epidemiological data suggest that the glucose response to an oral glucose tolerance test (oGTT) is a better predictor of heart disease risk than fasting glucose level. For instance, the Diabetes Intervention Study (DIS), a prospective trial of newly detected occurrences of Type 2 diabetes, reviewed health information for approximately 1100 adults over 11 years. The main outcome measures were myocardial infarction and death. The participants who died had significantly higher postprandial glycemia at baseline than those who did not. Interestingly, fasting blood glucose at baseline was not

associated with death. The researchers concluded that strict control of postprandial hyperglycemia is necessary to reduce the risk of MI and death in Type 2 diabetic individuals [76].

The above study, however, proves only that high postprandial glycemia predicts, not causes, cardiovascular complications and death. The STOP-NIDDM trial was an intervention trial in which the effects of altering postprandial glycemia were investigated. Started in 1995, it tested the effectiveness of the α-glucosidase inhibitor acarbose on the development of Type 2 diabetes. Acarbose specifically lowers postprandial glycemia by inhibiting the action of α-glucosidase, an enzyme required for the breakdown of carbohydrates to monosaccharides. Its effect is to slow down carbohydrate absorption, and therefore glucose appearance, into the circulation. Individuals receiving acarbose had a 25% less chance of developing diabetes than a placebo group [77]. They were also less likely to experience cardiovascular events and hypertension [78].

2.2.3 The Effects of Postprandial Hyperglycemia on Health

According to the World Health Organization, a blood glucose concentration above 11.0 mmol/L in the 2 hours after a 75g oral glucose tolerance test (oGTT) is considered hyperglycemia and thus represents their main diagnostic criteria for diabetes [79]. According to their recommendations, this post-challenge blood glucose level distinguishes individuals "with significantly increased premature mortality and increased risk of microvascular and cardiovascular complications." Blood glucose levels between 7.8 to 11.0 mmol/L are considered indicative of impaired glucose tolerance, and therefore increased risk of developing Type 2 diabetes and cardiovascular complications. Normoglycemia is defined as a post-challenge blood glucose concentration of 7.8 mmol/L and below. However, the WHO stresses that there is no threshold for predicting the risk of Type 2 diabetes and CVD such that post-oGTT blood glucose concentrations of approximately 5.50 mmol/L and above are linearly correlated with developing these diseases.

Postprandial hyperglycemia is detrimental to health because it causes oxidative stress [80, 81]. Oxidative stress can be defined as an imbalance between the production of oxidation products and the ability of antioxidant mechanisms to neutralize them, resulting in an excess of oxidation products [80]. Common oxidants such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), peroxynitrite ($ONOO^-$) and hydroxyl radical (OH^-) can cause vascular damage. Furthermore, hyperglycemia is particularly detrimental to the vascular system because the endothelial cells of the vascular endothelium are unable to regulate glucose uptake, allowing excess glucose to enter the cytoplasm [81].

Excess glucose can lead to the production of the above oxidation products, or reactive oxygen species, by a number of enzymes and pathways. One mechanism to explain the overproduction of reactive oxygen species (ROS) has been demonstrated by Brownlee *et al*. [72]. According to their research, high glucose concentrations within these cells results in the overproduction of superoxide by the mitochondrial electron-transport chain. Glucose is metabolized first through glycolysis and then the tricarboxylic acid cycle, producing electron donors. These are needed to create a proton gradient across the inner mitochondrial membrane via the electron transport chain. Excess glucose causes the overproduction of electron donors, which in turn increases the proton gradient across the mitochondrial membrane. Finally, a prolonged period of O_2^- production occurs when a gradient threshold level is reached. The result is the initiation of four pathways that lead to vascular damage: increased glucose entering the polyol pathway, increased production of advanced glycation end-products (AGEs), increased protein kinase C activity and increased flux through the hexosamine pathway [71, 81]. Indeed, it has been shown that diabetics have higher levels of ROS. Even typical levels of glycemia attained through an oGTT cause a decrease in plasma antioxidant capacity, indicating oxidative stress. In one study done by Ceriello *et al*., diabetic subjects were given two meals with different amounts of carbohydrate. Postprandial glycemia and susceptibility of subjects' LDL cholesterol to oxidation were measured after each meal. Oxidative modifications to LDL render it more atherogenic. It was reported that the susceptibility of LDL to oxidation was significantly higher after the meal that elicited the greatest hyperglycemia, confirming the hypothesis that hyperglycemia causes oxidative stress [82].

2.2.4 Dietary control of Postprandial Glycemia

Dietary approaches aimed at adequate glycemic control principally target the reduction of the overall glycemic response (blood glucose spikes and AUC) in conjunction with a reduction in insulin secretion. Related dietary approaches include changes of the type and amount of carbohydrate with preference for carbohydrates with slow or low availability. These carbohydrates maintain adequate postprandial glycemic control in terms of both 2-hour glycemia and postprandial spikes, without excessive rises in serum triglycerides and insulin and without reducing carbohydrate intake below the recommended daily amount [83].

One measure of the nutritional value of a carbohydrate food can be expressed by its Glycemic Index value. The Glycemic Index (GI) is a classification system for carbohydrate-rich foods based on their ability to raise blood glucose [84]. The GI value for a food is calculated by dividing a subject's glucose AUC for a 50-g carbohydrate test meal by that of the same subject's AUC for 50g glucose or white

bread, and multiplying the number by 100 [85]. Expressing the glycemic response of a food as a proportion of the glycemic response of a standard carbohydrate meal greatly reduces between-subject variation and creates a system that can be applicable to almost anyone. Experiments to determine the GI value of a food usually require a minimum of 10 subjects [86]. Many such experiments have been conducted, and as a result, the GI values of many carbohydrate-rich foods are known.

Carbohydrate foods that are absorbed quickly, such as white bread and cornflakes, cause a rapid spike in blood glucose and thus have high GI values according to the equation above. On the other hand, foods that are absorbed slowly and which promote slower, steadier increases in blood glucose have low GI values. Beans and pasta are examples of low-GI foods. The presence of dietary fiber and physical characteristics such as botanical structure help determine the GI of a carbohydrate. It should be noted, however, that GI cannot be predicted from the fiber content of a food.

This concept has proven instrumental in evaluating the preventative role of low-glycemic index foods in cardiovascular health [87] and in the development of diabetes [88] in both the healthy population and in individuals at risk. Both prospective observational and clinical trials have been undertaken to compare the effects of high-glycemic and low-glycemic diets on risk factors for diabetes and cardiovascular disease. Low-glycemic diets tend to be associated with lower blood lipids and glycated haemoglobin. Brand-Miller performed a meta-analysis of 14 trials and reported that low GI-diets reduced HbA1c in nine of the studies and reduced glycated proteins (HbA1c and fructosamine) by 7.4% of the starting values [89]. Further, prospective studies done by Stevens *et al* [90], Hodge *et al*. [91] and Schulze *et al.* [92] all showed that the risk of developing diabetes was significantly greater on a high-GI diet. This effect is not always consistent, however [93, 94]. Taken together, the results of clinical trials studying the effects of diet GI on glycemic control suggest a small but significant improvement on low-GI diets.

2.2.5 The Components of Salba affecting Postprandial Glycemia

There are several components of this grain that may lower postprandial glycemia; including its fiber, polyunsaturated fat and protein contents. Each of these nutrients has been repeatedly and separately shown to reduce glycemic responses. Together, they may act additively to provide Salba with an ability to lower postprandial glycemia.

2.2.5.1 The Effects of Carbohydrates and Dietary Fiber on Postprandial Hyperglycemia

Although 35% of Salba is carbohydrate, 34% of it in the form of fiber [25]. Thus, it contains only 1% available carbohydrate. As mentioned previously, 2.3% of the fiber is soluble and 32% is insoluble. Both types of fiber have beneficial effects on health and have been shown to lower postprandial glycemia, although the effects of soluble fiber on blood glucose have been more pronounced [95, 96]. The mechanisms put forth to explain fiber's ability to lower glycemia include slowing the rate of digestion of starchy polysaccharides in the stomach, slowing the rate of passage of the contents of the stomach into the duodenum, lowering the rate of hydrolysis of polysaccharides in the upper small intestine, lowering the rate of diffusion of carbohydrates in the small intestine and reduction of the rate of absorption of monosaccharides through the microvilli of the epithelial cells in the jejunum and upper ileum [86, 97, 98].

2.2.5.1.1 The Effects of Different Types of Fiber on Postprandial Hyperglycemia

2.2.5.1.1.1 *Soluble Fiber*

Soluble fibers are hygroscopic, meaning they have the ability to absorb liquids, and when in contact with liquids, expand to form a gel-like substance. Examples include gums and mucilages from oatmeal, barley and legumes, and pectin found in fruits such as apples and strawberries [29]. Because it absorbs liquids and expands in the stomach, soluble fiber slows the rate of gastric emptying. As a result, food is absorbed over a longer period of time, slowing the rate of glucose absorption and release into the bloodstream and thus reducing postprandial glycemia [29].

Extensive research supports the premise that soluble fiber significantly decreases postprandial glycemia [98, 99]. Furthermore, clinical studies have demonstrated enhanced glucose tolerance and increased insulin sensitivity in individuals consuming soluble dietary fibre supplements [95]. Soluble fibre has also been shown to blunt the increase in and insulin following a glucose load [100]. The majority of studies reviewed by Pilch found that soluble fibre intake results in an enhanced glucose tolerance and increased insulin sensitivity [29]. In one parallel study, Anderson *et al.* provided diabetic subjects supplements containing either psyllium or cellulose twice daily for 8 weeks [83]. Participants who ingested the psyllium, which contains mainly soluble fiber, experienced a 19.2% and 11.0% reduction in average lunch postprandial glycemia and fasting glucose serum total, respectively. Soluble fiber has

also been linked to reduction of blood lipids [101]. In the same study, serum total cholesterol and LDL cholesterol were reduced in the psyllium group by 8.9% and 13.0%, respectively.

2.2.5.1.1.2 *Insoluble Fiber*

Salba is composed of 32% insoluble fiber. Insoluble fibers are those that bind to liquids, and include celluloses and lignins. This type of fiber has been associated with reducing GI transit time, increasing fecal bulk, improving laxation and maintaining healthy intestinal and colonic pH levels [98].
It has been suggested that insoluble fiber has minimal effects on postprandial glycemia, gastric emptying and nutrient absorption [98]. For instance, Samra and Anderson demonstrated that varying the amount of insoluble fiber in a preload has no effect on postprandial glycemia [102]. Yet, Schenk *et al*. showed that insoluble fiber in cereal increases the rate of glucose uptake without affecting glucose absorption, resulting in lower postprandial glycemia [103]. Greater glucose uptake was achieved with a greater secretion of insulin in response to the insoluble fiber. Weickert *et al*. reported that consumption of 31g insoluble fiber for 3 days significantly improved whole-body insulin sensitivity compared to white bread control [104]. In another study, increased intake of insoluble fiber in the form of wheat fibre- and oat fibre-enriched bread over 24 hours reduced the glycemic response to a subsequent white bread meal by 31% and 32%, respectively [105]. Therefore, there is evidence to suggest that insoluble fiber affects glycemic responses.

2.2.5.1.2 The Effects of Fiber on Postprandial Glycemia: Mechanisms of Action

2.2.5.1.2.1 *Soluble Fiber: Mechanisms of Action*

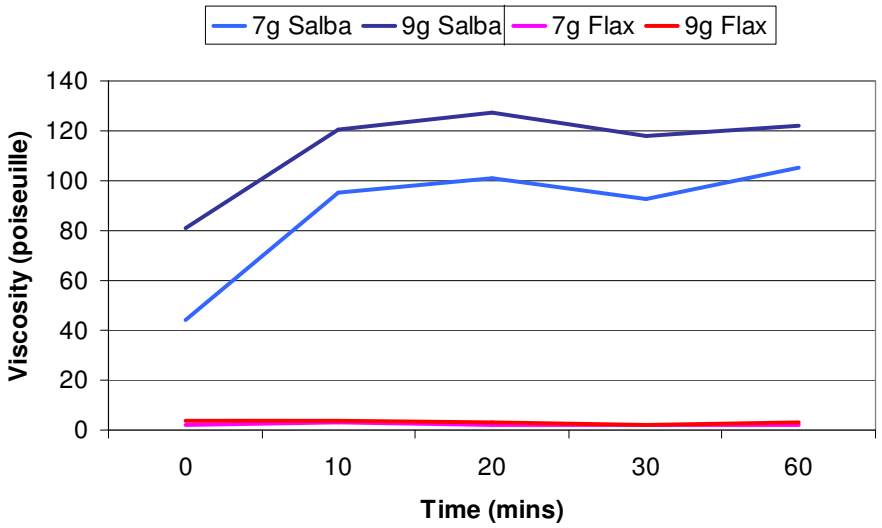
Viscosity is one of the most important rheological properties of soluble fiber, as it largely determines the health benefits of the fiber [106]. Viscosity is defined as a liquid's resistance to flow; it can also be described as a liquid's "thickness". Viscosity is positively correlated with a fiber's ability to improve glycemic control and lower blood lipids [107].
When viscous fiber interacts with the liquid contents of the stomach and intestines, it forms a gelatinous matrix and expands. This viscous mixture prolongs absorption of carbohydrates by slowing gastric emptying, and therefore slowing the release of glucose into the bloodstream [108]. The gelatinous fiber mixture may also trap ingested food, resulting in fewer nutrients being absorbed in a

given amount of time and thus less glucose released into circulation. Soluble fibre reportedly traps carbohydrates to slow their digestion and absorption, serving to prevent wide swings in blood sugar and insulin levels throughout the day [28]. Other mechanisms proposed to explain this phenomenon are resistance to the mixing action in the intestine, inhibition of enzyme activity and increased mucin production [98, 106, 109]. Lastly, the production of short-chain fatty acids may explain soluble fiber's ability to lower postprandial glycemia [98]. Butyrate, propionate and acetate are the short-chain fatty acids are by-products of soluble fibre fermentation in the colon, and there is evidence that diets enriched with short chain fatty acids reduce fasting glucose levels and postprandial glycemia [110, 111]. Some animal studies have found that the presence of acetate may result in a reduction in blood glucose possibly by inhibiting endogenous glucose production [112]. Additionally, it has been suggested that the production of acetate can lead to enhanced extrahepatic insulin secretion in the presence of raised blood glucose [113].

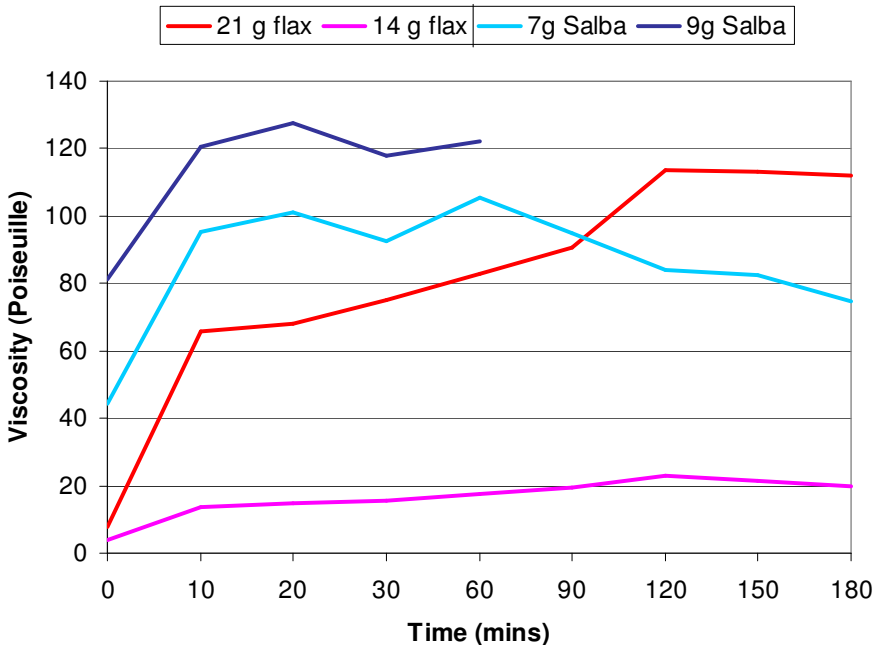
2.2.5.1.2.2 Viscosity of Flax versus Salba

Even though Salba contains only 2.3% soluble fiber, this fiber is especially viscous as evidenced by the comparison of the viscosity of ground Salba and ground flaxseed performed in our lab. Various amounts of each were added to 200 mL water, and the viscosity of both were measured using a Synchro-electric viscometer (Brookfield Ltd. Stoughton, MA, USA) at 22°C with a sheer of rpm 12 and with spindle E. Viscosity was measured at regular intervals for 1 to 3 hours. (Graphs 2.1 and 2.2) We found that Salba was approximately three times more viscous than an equal amount of flaxseed [114]. Interestingly, flax has on average 10g soluble fiber per 100g, making its soluble fiber content more than three times greater than that of Salba (2.3g per 100g). Therefore, when accounting for the difference in content, one gram of Salba has a comparable level of viscosity as approximately 9g flaxseed, a fact that may contribute to Salba's ability to reduce postprandial glycemia.

Graph 2.1 Viscosity of equal amounts of ground Salba and flax



Graph 2.2 Viscosity of different amounts of ground Salba and flax



2.2.5.1.2.3 Insoluble Fiber: Mechanisms of Action

Insoluble fiber may not delay gastric emptying as soluble fiber does, as a limited number of studies have demonstrated its glucose-lowering ability [98]. It may, however, lower postprandial glycemia by increasing glucose uptake into tissues. In the study done by Schenk *et al*. greater glucose uptake was achieved with a greater secretion of insulin in response to insoluble fiber [103]. Insoluble fiber also increases transit time in the small intestine, which may decrease the amount of food absorbed, lowering postprandial glycemia [115].

2.2.5.2 The Effects of Fat on Postprandial Glycemia

Thirty-three percent of Salba is fat, a factor contributing to its glucose-lowering ability. Research has demonstrated that adding fat to a carbohydrate meal significantly lowers postprandial glycemia [116, 117]. The addition of fat to a carbohydrate meal does not affect postprandial glycemia in a linear fashion, however. It has been shown that the greatest relative reduction in postprandial glycemia is seen for small amounts of fat [86, 118]. When the ratio of fat to carbohydrate is approximately 0.05-0.2g fat to 1g carbohydrate in a meal, fat reduces blood glucose by the greatest proportion. In most meals with carbohydrate and fat, fat comprises 20-45% of the total energy [86]. In the experiments conducted for this thesis, the ratios of fat to carbohydrate in the experimental meals of both studies were 0.07, 0.13 and 0.18 for low, intermediate and high doses, respectively. This represents a percentage of calories from fat of 11%, 18% and 24 %, respectively.

It must be noted that the AUC of a glucose response for a carbohydrate and fat meal may not portray the full extent that postprandial glycemia is altered. Adding fat to a carbohydrate meal may also change the shape of the glucose response curve since not only does fat slow the rise in blood glucose, but it slows the fall as well [86]. Therefore, the area under the curve for glucose could be quite similar for carbohydrate foods with or without fat, but the shapes of the curves different. For instance, Cunningham and Read demonstrated that adding fat to a soup meal delayed the occurrence of the peak glucose level [119]. Thus, it has been shown that the addition of fat to a carbohydrate-rich meal lowers the peak rise of glucose, suggesting that fat stabilizes glucose levels in the blood.

2.2.5.2.1 The Effects of Different Types of Fat on Postprandial Glycemia

Various studies have demonstrated that different types of fat affect postprandial glycemia differently. For instance, it appears that postprandial glycemia decreases as the degree of unsaturation increases [120, 121]; however this effect isn't consistent [122, 123]. Joannic *et al* tested glucose and insulin responses to four meals in which the ratio of monounsaturated fatty acids and polyunsaturated fatty acids, and the type of carbohydrate (potatoes or par-boiled rice) were varied [124]. The two kinds of fat used were a mixture of 70% high-oleic sunflower oil and 30% rapeseed oil (high MUFA to PUFA ratio), and a mixture of 60% sunflower oil and 40% soybean oil (low MUFA-PUFA ratio). Blood samples were taken every 30 mins for 3 hours post-consumption. Results showed that the glucose AUC's for both types of fat mixtures did not differ significantly. However, at 30 mins, the glucose response was significantly lower for both PUFA meals compared to the two MUFA meals. Thus, this study demonstrated that the degree of fat saturation affected the early postprandial glucose response [124]. Additionally, Gatti *et al.* established that the co-ingestion of saturated fat and white bread did not affect postprandial glycemia, while olive and corn oil reduced it [120].

Conflicting results were found in a study testing the effects of potato with either butter or olive oil. Potatoes eaten with 100 g butter significantly reduced the blood glucose response area, while potato with olive oil (40 and 80g) or 50g butter had no affect [122]. Further, MacIntosh *et al*. studied the glucose responses to three types of fat: butter, Sunola oil (MUFA) and sunflower oil (PUFA). No effect of the degree of saturation on glycemic responses was found. The fact that MacIntosh *et al.* studied only males, administered a different amount of fat and measured blood samples only 2 hours post-consumption may explain the discrepancy between their findings and those of Joannic *et al.*

The effects of the type of fat on postprandial glycemia may be explained in part by the binding affinity of intestinal fatty acid binding protein (FABP2)[86, 121]. Fat is first hydrolysed by pancreatic lipase to fatty acids and monoglycerides, which are absorbed into enterocytes in the small intestine. Here they are reassembled into triglycerides that are incorporated into chylomicrons. Some of the absorbed fatty acids enter the portal circulation as free fatty acids, however, and go straight to the liver. Different free fatty acids are absorbed in different amounts, partly due to differences in their affinities for intestinal FABP2. A high level of free fatty acids in the portal vein is purported to increase hepatic glucose output [86].

Human FABP2 has the highest affinity for long-chain fatty acids such as palmitic, stearic, oleic and linoleic acids [121]. It appears that binding affinity decreases as the chain length decreases [121]; however this effect isn't consistent in rats [125]. Nonetheless, the pattern of incorporation of fatty acids

into chylomicrons may explain why postprandial glucose after meals with butter are higher than those with olive and maize/safflower oils. Butter contains roughly 25% of its fat as short- and medium-chain fatty acids, while olive oil has about 75% oleic acid and safflower oil about 75% linoleic acid.

2.2.5.2.2 The Effects of Fats on Postprandial Glycemia: Mechanisms of Action

Prolonging gastric emptying causes a reduced rate in carbohydrate absorption, resulting in lower postprandial glycemia [116, 119, 126]. Although it is generally accepted that fat delays gastric emptying, some researchers argue that this characteristic is not specific to fat. Instead, it is a product of additional nutrients [86].

Fat is proposed to delay gastric emptying by stimulating gut hormones such as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). However, carbohydrate and protein also increase these hormones to the same extent. Furthermore, one study that used 13C-labelled carbohydrate demonstrated that adding fat to a carbohydrate meal reduced postprandial glycemia without a change in the rate of appearance of the labelled carbohydrate in the blood. Therefore, delayed gastric emptying may not be an adequate explanation for fat's ability to lower postprandial glycemia [86].

In one study by Collier *et al*., butter was added to either potatoes or lentils [127]. The postprandial insulin response did not differ significantly from the responses to the carbohydrates alone. However, the glycemic responses to the fat + carbohydrate meals were significantly decreased. When fat was consumed with the meal, a lower amount of glucose required the same amount of insulin, suggesting the potentiation of the insulin response. On the other hand, a decrease in insulin sensitivity is also possible.

Some researchers report that fat increases insulin secretion [124, 128]. For instance, Gannon *et al*. found that the insulin response area to a starch + fat meal was significantly higher than that of a starch-only meal [117]. Yet, some research demonstrates that while ingestion of fat does not result in significant differences in insulin secretion, differences in glucose levels are evident as mentioned above. This suggests that the ability of insulin to instigate glucose absorption may be altered when fat is added to a carbohydrate meal. More specifically, ingestion of fat causes insulin insensitivity [127]. Studies that don't show a difference in insulin responses between carbohydrate and carbohydrate + fat meals often show lowered postprandial glycemia for the fat meal, suggesting that the same amount of insulin was needed for less glucose. One study even hypothesized that the effectiveness of insulin

depends on the type of fat ingested [129]. Research in this area is inconsistent, however, as insulin responses differ in different studies.

2.2.5.3 The Effects of Protein on Postprandial Glycemia

The results of experiments that add protein to carbohydrate meals are inconsistent: several studies show a reduction in glycemic response, while others show an increase[86]. Wolever reasons that glycemic responses vary to such a great degree because there are many possible mechanisms by which protein alters glucose metabolism and because they depend on different the rates of digestion and absorption of individual amino acids[86].

Nonetheless, Gannon *et al.* measured the glucose and insulin responses to glucose alone versus glucose and lean beef, turkey, gelatin, egg white, cottage cheese, fish or soy in individuals with Type 2 diabetes. All glucose and protein meals resulted in a significantly lower glucose AUC than the glucose meal, except when egg white was used. Furthermore, the insulin AUC was greater for all protein meals. A previous study done by the same authors also demonstrated a synergistic effect on insulin release when glucose and protein are consumed together [130].

2.2.5.3.1 The Effects of Different Types of Protein on Postprandial Glycemia

Different types of protein affect insulin secretion to different degrees. For instance, Gannon *et al* reported that protein from cottage cheese stimulated maximum insulin secretion, while protein from egg whites stimulated insulin secretion to the lowest extent[131]. It is presumed that egg white is very poorly digested and therefore increases insulin only slightly compared to egg white. Although the increase in insulin secretion does not occur in a linear manner, the reduction in postprandial glycemia does. Therefore, insulin cannot completely account for the lower glucose levels. Indeed, Wolever reports that the insulin response to protein is responsible for approximately 30-40% of the glucose response.

2.2.5.3.2 The Effects of Protein on Postprandial Glycemia: Mechanisms of Action

It is generally accepted that protein's ability to lower postprandial glycemia is caused by delayed gastric emptying [132]. As well, the ability of amino acids to promote insulin secretion has been put forth as the main cause of the increase in insulin seen after consumption of protein [133], and therefore

another reason why protein lowers postprandial glycemia. Yet, the ingestion of protein has also been shown to increase postprandial glycemia when added to carbohydrate meals [134], and when added to carbohydrate and fat meals as well [135]. There are a few mechanisms by which protein can increase glycemic responses; namely, the stimulation of glucagon secretion, conversion of amino acids to glucose and increase in insulin resistance caused by amino acids. It is important to note that when protein is ingested alone, it has no or very little effect on blood glucose in people with or without Type 2 diabetes [136, 137].

Given the conflicting results from protein and carbohydrate meal studies, it is difficult to predict the effects of the protein in Salba on postprandial glycemia. Regarding the control meals, it would be expected the protein used (egg white) would not affect postprandial glycemia since this protein has been shown to have no effects on blood glucose.

2.2.5.4 Antioxidants and Hyperglycemia

Antioxidants do not have a postprandial glycemia-reducing effect. However, they can help alleviate ill effects of hyperglycemia by combating oxidative stress. As mentioned previously, hyperglycemia increases the production of ROS [80, 138]. This, in turn, causes the body to be more susceptible to a variety of diseases. For instance, a state of oxidative stress increases the amount of oxidized LDL cholesterol, which is a risk factor for CVD. Therefore, although antioxidants cannot prevent hyperglycemia, they can help reduce the detrimental effects by binding to and deactivating harmful ROS [139].

2.3 The Short-term Regulation of Appetite

With the current high rates of obesity and obesity-related complications, increasing attention has been given to the control of food intake as a preventative measure. It is presumed that the effects of short-term appetite reduction act cumulatively to decrease total food intake over a prolonged period of time, in turn preventing excessive caloric consumption and weight gain [140]. Decreasing appetite is a very topical issue, intriguing both the research community and general public alike.

This thesis focuses on postprandial feelings of appetite. In the research setting, the question remains as to whether subjective feelings of appetite influence food intake. Some studies have shown that intentions to eat and ratings of hunger correlate with actual food consumption, while others show no correlation [141]. Nonetheless, the measurement of subjective appetite is widely used in nutrition research and is accepted as a valid measure by the medical community [142-145].

The regulation of appetite is a multifaceted process that although extensively researched, is not yet completely understood. Short-term hunger and satiation are affected by numerous psychological, mechanical and physiological factors [145-151]. Of particular interest to this thesis are the anorectic gastrointestinal hormones that are released in response to a meal, namely cholecystokinin (CCK), pancreatic polypeptide, peptide YY and glucagon-like peptide-1 (GLP-1) that act as negative feedback signals to the satiety centres of the brain [147]. When released from enteroendocrine cells in the gut mucosa, these hormones can communicate with brain regions via the circulation and afferent nerve fibers to signal food intake to the central nervous system [146-148]. CCK was the first anorectic hormone to be discovered. It is released postprandially and its concentration in the circulation can remain elevated for up to 5 hours after a meal. CCK inhibits food intake in both humans and rats and initiates gallbladder contraction and secretion of pancreatic enzymes. GLP-1 is another anorectic hormone that is released in response to a meal. The amount released is dependant on the amount of calories ingested, and it modulates gastric emptying and acid secretion. Infusion of GLP-1 reduces food intake and increases feelings of satiety.

Ingested food is sensed by vagal afferent fibers in the mucosa, while food volume is detected by vagal afferent nerves in external muscle layers of the gut [146]. Food volume and intragastric pressure are both important factors in the initiation of satiety signalling, and may be the main cause of the satiating effects of soluble dietary fiber. As well, circulating glucose, lipids and amino acids all affect feelings of appetite and food intake. The effects of protein and fat on appetite are particularly relevant to this thesis.

The Glucostatic Theory, although not fully validated or proven, is one of the most well-known and researched hypotheses linking appetite and macronutrient intake. Proposed by Mayer in 1951, it is a homeostatic theory of hunger and states that the body has mechanisms in place to regulate glucose levels and feeding behaviour [152]. Although other nutrients affect eating behaviour as well, glucose is deemed to be of particular importance due to its key role as an energy source for the central nervous system. If the body detects hyper- or hypoglycemia, signals are initiated to bring the glucose level back to an acceptable range. For instance, when blood sugar is low, signals are sent to the hunger centres of the brain to initiate feeding in an attempt to raise plasma glucose [152-154].

Many studies support the Glucostatic Theory by demonstrating associations between glucose levels and feeding behaviour or hunger in rats [155, 156] and humans [157]. Campfield and Smith found that transient declines in blood glucose resulted in food seeking and meal initiation in rats [155]. Pittas *et al*. studied the associations between interstitial glucose and energy intake, desire for a meal, self-reported hunger and satiety in free-living nonobese women. Absolute interstitial glucose values up to 35 mins before meal initiation were significant predictors of food intake such that low absolute glucose values preceded meal initiation. Blood glucose lags interstitial glucose by 10 mins; thus, the authors hypothesized that blood glucose levels up to 25 mins before meal initiation are negatively correlated with subsequent food intake [158].

In a study by Gielkens *et al*., subjects received intravenous infusions of glucose, insulin or saline (control). The authors found that hyperglycemia (15 mmol/L) induced significantly lower prospective intentions of feeding and feelings of hunger compared to control or hyperinsulinemic conditions [157].

In a study done by Anderson *et al*., subjects consumed a drink containing polycose, sucrose, glucose, a glucose-fructose mixture or sucralose control. Over the next hour, blood glucose and self-reported hunger were measured at various time points. Food intake after 60 mins was measured via an *ad libitum* pizza meal. It was reported that glucose iAUC was negatively correlated with energy intake [144].

However, Wolever *et al*. found no correlation between blood glucose and satiety as measured by VAS for 2 hours postprandially [142]. Participants consumed the same white bread meal on separate occasions and the postprandial glucose iAUC was measured for each session. The satiety scores for the meals eliciting the greatest and smallest AUC's were compared, and it was reported that they did not differ significantly. The authors suggest that the increased satiety after low-GI foods must occur through a mechanism that does not rely on blood glucose level. In another study, Freeland and Wolever measured postprandial glycemia and food intake after low- and high-fiber cereals and found that blood

glucose responses were not correlated with energy intake [159]. Other studies have also failed to demonstrate a link between blood glucose and satiety [160, 161].

There is still debate as to whether it is a change in blood glucose or the absolute blood glucose level that influences eating behaviour. Pittas *et al.*'s study provides support for absolute levels being the determinant of food intake, while Campfield and Smith reported that it was changes in glucose level that affected it. This issue will be discussed further in a following section.

2.3.1 The Components of Salba potentially affecting Appetite

Salba's components may exist in favourable proportions and amounts to increase satiety. Firstly, Salba is composed of 34% dietary fiber, a nutrient implicated in reduced feelings of hunger and lower risk of obesity. As well, the type of fat in this grain has been reported to be satiating. In addition, with a calcium content of 770mg per 100g, this grain is exceptionally high in calcium. Intracellular calcium has a regulatory role in fat metabolism by influencing lipolysis, fat oxidation, and lipogenesis; all processes that may influence appetite regulation [162]. Lastly, Salba contains a significant proportion of protein, the most satiating macronutrient [163]. We thus hypothesize that all these factors may act additively to promote satiety, in particular Salba' fiber, polyunsaturated fat and vegetable protein contents.

2.3.1.1 The Effects of Carbohydrates and Dietary Fiber on Appetite

2.3.1.1.1 *Dietary Fiber*

Both soluble and insoluble fiber have been shown to reduce appetite and/or subsequent food intake [100, 164, 165]. Howarth *et al*. analyzed the results of over 20 studies in which the effects of fiber on hunger and satiety were measured [115]. In the majority of these studies, the addition of fiber to the diet caused either a significant or nonsignificant increase in satiety between meals and/or a decrease in hunger relative to control. No differences were found for the effects of soluble versus insoluble fibers. The two types of fiber decrease hunger by different mechanisms. Unlike insoluble fiber, soluble fiber becomes viscous when mixed with liquid, leading to mainly mechanical effects on appetite. Increased gastric distention due to the swelling of soluble fiber creates a feeling of fullness by activating stretch receptors within the gut walls and initiating afferent vagal signals [115]. Soluble fiber also delays gastric emptying, meaning food remains in the stomach longer, which creates a feeling of fullness for a longer amount of time [115].

Soluble fiber-rich foods are exceptionally low density due the fact that fiber is not digested and that the soluble component swells in the stomach. Many studies done by Rolls *et al* have proven that high volume foods are more satiating than low volume foods [166-168]. For instance, incorporating air into a beverage resulted in less subsequent energy intake than the beverage alone. Soluble fiber influences satiety by metabolic pathways as well, by possibly stimulating the secretion of gut hormones that signal satiety [115].

Lowered postprandial glycemia, a metabolic effect, is yet another mechanism that has been suggested to increase feelings of fullness. Soluble fiber slows gastric emptying and traps carbohydrates, slowing their digestion and absorption. This, in turn, is hypothesized to increase satiety by preventing sudden drops and wide swings in blood sugar levels [28, 169], signals that would normally trigger hunger as explained in the Glucostatic Theory. In line with these hypotheses, it was found that subjective feelings of appetite were greater after a greater consumption of pectin, a soluble fiber, than the same meal without it [170].

On the other hand, insoluble fiber increases food transit time in the small intestine, which may result in decreased food absorption. Thus more undigested food particles reach the distal intestine, which is proposed to increase the secretion of satiety hormones [171]. It has also been reported that increased levels of insoluble fiber cereal decreased food consumed at an *ad libitum* meal in men [102].

2.3.1.1.2 Low GI Carbohydrates

Salba does not have a GI value *per se*, as it contains no carbohydrate. Yet, when added to a carbohydrate meal, it has the ability to lower the meal's glycemic response. Low Glycemic Index (GI) foods have received much attention in recent years for their purported effects on hunger. Researchers theorize that a consistently stable, moderate level of plasma glucose signals an adequate supply of glucose and as a result, hunger signals are not initiated. However, high-GI foods cause an immediate and large increase in blood glucose levels. In an attempt to normalize the blood glucose level, a great amount of insulin is released, leading to the rapid removal of glucose from the blood. The counter-regulatory response may overcompensate, resulting in hypoglycemia. Hunger signals are then initiated. It follows that since low-GI foods promote stable blood glucose levels and prevent precipitous declines into hypoglycemia, they would also prevent hunger [28].

Results from studies evaluating the effects of GI on satiety have been inconsistent. Roberts evaluated the results of studies measuring satiety, hunger and/or food intake after low-GI and high-GI preloads [172]. These studies controlled for caloric value, energy density and palatability of the test meals.

Although there were no significant differences for satiety and hunger as measured by visual analog scales, all five studies demonstrated lower subsequent energy intake following the low-GI meals (three were significant). Roberts also reported that a meta-analysis of these experiments revealed an 81% larger energy intake after high-GI meals than after low-GI meals.

It is postulated that since low-GI foods are digested more slowly, more undigested starch reaches the ileum [173]. It is believed that the presence of starch in this region promotes the release of satiety-signalling hormones such as GLP-1[171]. Another theory to explain the effect of low-GI carbohydrates on appetite is the change of fuel source availability depending on the GI-value. Consumption of a high GI-meal may promote the uptake of glucose and fatty acids while decreasing lipolysis [174, 175]. Thus levels of these macronutrients decrease in the circulation, and since this represents a decrease in circulating metabolic fuels, hunger is initiated.

Ludwig concluded from 20 GI studies that low-GI meals consistently lower appetite and/or food intake [176]. Unfortunately, many of these studies failed to control for confounding factors [86]. For instance, in some experiments the test meals contained different amounts of protein or fiber, two components that can affect hunger. Another study included in the meta-analysis tested meals with different glycemic loads, but not GI values. Therefore, although one cannot dismiss the theory, more research must be conducted. With this in mind, it can be theorized that the consumption of Salba may help increase satiety since its components promote the moderate and stable release of glucose into the circulation and the induction of satiety signals.

2.3.1.2 The Effects of Fat on Appetite

Researchers consider fat to be the least satiating nutrient in the short-term [140], mostly due to its high energy density and its palatability [87, 177]. Studies have shown that high-fat foods lead to passive overconsumption compared to lower-fat, less energy-dense foods [178]. Due to its effects on gastric emptying, however, fat may be satiating for a longer period of time than the other macronutrients. Also, it has been demonstrated that dietary fat or the products of its digestion are more effective stimulators of CCK release than carbohydrate [179].

Salba contains mainly polyunsaturated fat, which has been reported to induce greater satiety than other types of fat. For instance, the degree of saturation of fat appears to affect satiety. Two studies conducted by Lawton *et al*. tested the effects of three types of fat incorporated into a meal. Fat A consisted of mainly monounsaturated fat (oleic blends), Fat B polyunsaturated fat (linoleic blends) and Fat C saturated fat (stearic-oleic blends). Satiety was measured with visual analog scores, food intake

during an *ad libitum* meal and 24-hr food intake diaries[180]. The results showed that the polyunsaturated fat meal reduced appetite to the greatest extent. The authors hypothesized that the effects of fats on satiety depend on whether they are oxidized or stored. Friedman has suggested that when a fat is oxidized, it causes more satiety signals [181]. Saturated fat is not oxidized to fuel sources as readily as polyunsaturated fat [182], which would lend support to the results of Lawton *et al*..

2.3.1.3 The Effects of Protein on Appetite

Protein is the most satiating of macronutrients, and research has demonstrated that consumption of protein reduces both appetite and food intake compared to consumption of carbohydrate [140, 183]. Although carbohydrates, and especially simple carbohydrates, provide the most satiation immediately after consumption [184], protein provides feelings of fullness for prolonged periods of time. Thus in theory, Salba consumption may reduce feelings of satiety and food intake for a period of time beyond those of the effects seen from dietary fiber and carbohydrate when added to a carbohydrate meal. Evidence exists for the short-term, 24-hour and long-term satiating effect of protein [163]. For instance, greater satiety and GLP-1 levels were seen after a high-protein dinner compared to an adequate-protein dinner [185]. In one long-term study by Johnston *et al*., participants were put on either a high-protein/low-fat diet or a high-carbohydrate/low fat diet. Those in the high-protein group reported feeling more satiated during the first 4 weeks of the study [186]. Lejeune *et al*. studied the effects of a high protein versus an adequate protein diet on healthy females [187]. The HP diet contained 30% protein, 40% carbohydrate and 30% fat, while the AP diet contained 10% protein, 60% carbohydrate and 30% fat. The experiments were conducted in a respiration chamber, and diet-induced thermogenesis, sleeping metabolic rate and activity-induced energy expenditure were monitored. Twenty-four hour satiety was also measured via VAS. The authors reported that the high protein diet resulted in significantly greater satiety AUC. Diet-induced thermogenesis was also significantly greater in the HP condition. Subjects in another study reported siginificantly higher feelings of satiety 30 and 120 mins after consuming a lunch with 25% protein versus one with 10%. Satiety AUC over 2 hours was significantly higher in the former condition, as well [188].

Westerterp-Plantenga *et al*. suggest that diet-induced thermogenesis may be a factor in causing satiety [185]. It is theorized that increased oxygen consumption occurs during increased energy expenditure, and that this oxygen deprivation creates feelings of satiety [163]. Additionally, there is evidence that different protein sources elicit different levels of energy expenditure, and therefore perhaps satiety. For instance, it was reported that animal protein caused a 2% higher energy expenditure than vegetable

protein [163]. Thus, the protein in Salba may not promote as much satiety as would be expected from an animal source. Nonetheless, it can be hypothesized that the addition of vegetable protein to a carbohydrate-rich meal would be expected to lower appetite to a greater extent than an equicaloric amount of added carbohydrate.

3 Rationale and Objectives

3.1 Summary and Rationale

Salvia hispanica L., also known as Salba, is an ancient oil-rich grain with a unique and superior composition. Although consumed for centuries, little clinical research has been conducted to examine its potential effects on health. Research by Vuksan *et al.* suggests that Salba may decrease risk factors for CVD in Type 2 diabetes. Diabetic individuals who supplemented their CDA-recommended diet with 37g of Salba a day for a month experienced lower blood pressure, coagulation and low-grade body inflammation. As well, results from two studies demonstrate a possible role for Salba in the reduction of visceral adiposity, and perhaps, therefore, the risk of obesity. Vertommen *et al.* found a significant reduction of waist circumference in healthy individuals consuming Salba for one month. That there was no significant decrease in body weight is a possible indication that fat mass was preferentially lost. Salba has also been shown to reduce visceral adiposity in rats. Animals fed a sucrose-rich diet supplemented with Salba experienced lower visceral adiposity than those on a maize control diet in a 5-month study by Chicco *et al.* [5].

The promising results of these studies with Salba suggest a possible ability of this grain to reduce risk factors for CVD and obesity. More research is thus warranted to determine both the acute and long-term effects of this grain. With regards to Salba's acute effects, the reduction of postprandial glycemia may explain the results seen in aforementioned long-term studies, as hyperglycemia has been shown to trigger biochemical cascades related to atherosclerosis and CVD.

We theorize that the smaller blood glucose responses from consuming Salba may have promoted lower blood pressure, coagulation factors and body inflammation by the reduction of physiological pathways triggered by excess glucose. Postprandial hyperglycemia has been demonstrated to promote oxidative stress, which in turn affect endothelial function and LDL oxidation [71, 80, 138]. As well, we hypothesize that a reduction of appetite may have contributed to the decrease in waist circumference seen in Vertomment *et al.*.We thus investigated the acute effects of Salba in various dosages on postprandial glycemia and subjective appetite. We also employed whole and ground forms of this grain, as it is commercially available as ground and whole yet a comparison of the effects of these forms has not been assessed specifically. We began examining the possible relationship of acute and long-term effects in a series of acute studies measuring the effects of a Salba-enriched carbohydrate meal on postprandial glycemia and appetite, two factors that may play a role in the development and severity of obesity and CVD.

3.2 Hypothesis and Objectives

The main objective of this thesis is to evaluate the effects of Salba on postprandial hyperglycemia and appetite, two factors that have been suggested to play a role in the development of Type 2 diabetes, CVD and obesity.

3.2.1 Specific Objectives

3.2.1.1 Study 1

1. To measure the effects escalating doses of Salba added to white bread in reducing postprandial glycemia in healthy individuals
2. To measure the effects escalating doses of Salba added to white bread in decreasing subjective appetite in healthy individuals

3.2.1.2 Study 2

1. To determine the effects of escalating doses of ground vs. whole forms of Salba added to white bread on postprandial glycemia in healthy individuals
2. To determine the effects of escalating doses of ground vs. whole forms of Salba added to white bread on subjective appetite in healthy individuals

3.2.2 Hypotheses

3.2.2.1 Study 1

1. Salba will lower postprandial glycemia in a dose-dependent manner when added to white bread
2. Salba will decrease subjective appetite in a dose-dependent manner when added to white bread

3.2.2.2 Study 2

1. Salba-enriched white bread will lower postprandial glycemia to a significantly greater extent than equicaloric fat-, protein- and carbohydrate-matched white bread.
2. Salba-enriched white bread will decrease subjective appetite to a significantly greater extent than equcaloric fat-, protein- and carbohydrate-matched white bread.
3. Ground and whole forms of Salba will have comparable effects on both postprandial glycemia and subjective appetite.

4 Study 1: The Effects of Escalating Doses of Salvia hispanica L. (Salba) on Postprandial Glycemia and Subjective Appetite in Healthy Individuals

4.1 Abstract

Objective: To assess the effects of escalating doses of the whole grain Salba on postprandial glycemia and satiety.

Methods Using an acute randomized, double-blind, crossover design, 12 healthy individuals (7M;5F;BMI 22..2 ± 1.3 kg/m^2) received 0, 7, 15 or 24 g of Salba baked into white bread. The control (0g) was repeated twice. All meals contained 50 g of available carbohydrates. Thus, subjects consumed 5 different meals with at least 2 days between visits. Fingerprick blood samples and ratings of appetite scores on a 100 mm visual analog scale were taken at fasting and 15, 30, 45, 60, 90 and 120 mins post-consumption.

Results iAUC was reduced by approximately 2% for one gram of Salba. Glucose iAUC was negatively correlated with dose of Salba (r=-0.47, p=0.001). The highest and intermediate doses of Salba resulted in a reduction in blood glucose iAUC of 44% and 25% (p=0.002), respectively. Subjective appetite was also negatively correlated with dose of Salba (r=-0.3, p=0.043). The appetite iAUC for low, intermediate and high doses were lower than control by 54.0%, 62.1% and 66.8%, respectively, but these reductions did not reach significance. However, appetite for the high and intermediate doses at 90 mins (p = 0.044) and intermediate dose at 120 mins (p = 0.044) were significantly lower than control.

Conclusions Addition of the whole grain Salba to white bread lowers postprandial glycemia and subjective appetite, two possible factors that may help reduce the risk of obesity and CVD. Further research is warranted to assess the effects of Salba on long-term reduction of cardiovascular risk factors, carbohydrate metabolism and control of body weight.

4.2 Introduction

Recommendations for various dietary interventions have been attempted to reduce the risk of obesity, Type 2 diabetes and CVD. One suggestion is the reduction of postprandial hyperglycemia. Growing evidence on the independent predictive role of 2-hour postprandial glycemia for cardiovascular disease suggests that interventions should target this risk factor. Explanations of the risk of cardiovascular disease and diabetes linked to postprandial glycemia suggest that endothelial and beta cells, both freely

permeable to glucose via GLUT- 2 and 1, respectively, are susceptible to the excessive oxidative stress resulting from increased glucose uptake. Oxidative stress, in turn, increases risk factors for CVD such body inflammation and coagulation. Supplementing the diet with certain functional foods, such as whole grains, has been suggested as a dietary intervention to lower postprandial glycemia.

Due to the key role of obesity in the development of both cardiovascular disease and Type 2 diabetes, nutrition research has increasingly focused attention on appetite regulation. Researchers theorize that certain functional foods may help reduce the risk of obesity. With the presumption that lowered appetite would translate to less food intake [140], investigation of the ability of certain foods to lower appetite could be valuable in the prevention of obesity and CVD.

Evidence suggests that phytochemicals naturally-occurring in whole grains have the ability to lower postprandial glycemia and appetite. Phytochemicals such as fiber, minerals, unsaturated fat and antioxidants and nutrients such as vegetable protein may separately promote such effects. Here we investigate the ability of the novel whole grain *Salvia hispanica L.* (Salba) to lower postprandial glycemia and reduce appetite when added to a carbohydrate meal. Salba has a composition superior to commonly-consumed whole grains and oily seeds, as it contains comparable amounts or more fiber, omega-3 fatty acids, minerals, antioxidants and protein. In addition, the possible beneficial effects of Salba consumption such as decreased blood glucose and appetite may provide mechanisms to explain the results of previous Salba studies showing decreased blood pressure, low-grade body inflammation, coagulation factors and adiposity. These represent possible risk factors for Type 2 diabetes and CVD.

4.3 Methods

4.3.1 Participants

A total of 12 healthy individuals (7M; 5F; Age: 30.2 ± 3.6 years; BMI 22.2±1.3 kg/m^2) participated in the study, as typical G.I. studies employ from 10-12 participants [86]. All participants were healthy, between 18-66 years old and clinically euthyroid with normal hepatic and renal function. Those who were pregnant, suffered from gastrointestinal or metabolic diseases, or regularly ingested fiber supplements were excluded. All participants gave written informed consent, and the study was approved by the St. Michael's Hospital Research Ethics Board.

4.3.2 Treatments

There were five experimental meals consisting of 50g carbohydrate servings of white bread with 0, 7.3, 15.6 or 24g of ground Salba added. The lowest dose, 7.3g, contains the American Heart Association's daily minimum recommended intake of omega-3 fatty acids. The bread without any Salba comprised the control meal and was served twice. All breads were prepared on-site with a Black & Decker® All-In-One Pro™ Breadmaker (Towson, MD, USA). Two-hundred and fifty millilitres of water was served with each meal. Please see Table 4.1-4.3 for percentage of calories per macronutrient, bread ingredients and caloric values of experimental breads.

Table 4.1 Nutritional Information for Experimental Breads

	Control	Low Dose	Intermediate Dose	High Dose
Serving Size	100g	110g	117g	126g
Calories	232 kcal	260 kcal	292 kcal	324 kcal
Fat	1.0g	3.4g	6.0g	9.0g
Carbohydrate	52.6g	55.0g	57.9g	60.7g
Fiber	2.6g	5.0g	7.9g	10.7g
Available Carbohydrate	50.0g	50.0g	50.0g	50.0g
Protein	9.5g	3.4g	6.0g	9.0g

4.3.3 Experimental Design

The study consisted of a double-blind, placebo-controlled, randomized block design in which subjects underwent five 2.5-hour sessions separated by a washout period of at least 48 hours. Subjects visited the Risk Factor Modification Centre in the morning after having fasted for 10-12 hours overnight and engaging in normal eating and exercise habits the preceding day. They had an initial fasting finger prick blood sample taken and completed a subjective appetite questionnaire in the form of a 100mm visual analog scale (VAS). The experimental meal was then served and subjects were required to finish the meal within 15 mins. Finger prick blood samples were taken at 15, 30, 45, 60, 90 and 120 mins postprandially. Subjects also completed the appetite VAS at these times. For the duration of the study

session, subjects remained at the clinic and were instructed not to eat or drink and to keep physical activity to a minimum.

4.3.4 Blood Glucose Analysis

Capillary blood samples were obtained using sterile single-use lancets. Two to three drops of capillary blood were collected in plastic flat-bottomed 5ml tubes with a push cap containing a small amount of sodium fluoride and potassium oxalate as an anticoagulant and preservative. The blood samples were placed in a -20ºC freezer for a maximum of 5 days until the analysis of whole blood glucose. Capillary blood glucose was measured by the glucose oxidase method using a YSI 2300 STAT Plus Glucose & Lactate Analyzer™ (Yellow Springs Instruments, Yellow Springs, OH, USA).

4.3.5 Measurement of Appetite

Four unipolar visual analogue scales were used for each appetite assessment. For each of the four questions, subjects indicated their response by drawing a vertical line along a 100 mm horizontal line that was anchored by two statements. The questions were “How strong if your desire to eat?”, “How hungry do you feel?”, “How full do you feel?” and “How much do you think you could eat now?” Subjects’ ratings were converted to numerical values by measuring the distance between the left anchor and their drawing. A combined appetite score for each appetite assessment was computed with the following formula:

$$[\ Q1 + Q2 + Q4 + (100 - Q3)\ ] / 4$$ [141]

4.3.6 Study Variables

The primary variables for this study were the mean iAUC’s for blood glucose and the mean incremental change from baseline in blood glucose at 15, 30, 45, 60, 90 and 120 min postprandially. The secondary variables were the mean iAUC’s of satiety scores and the incremental change in appetite from baseline at the same time points.

4.3.7 Statistical Analysis

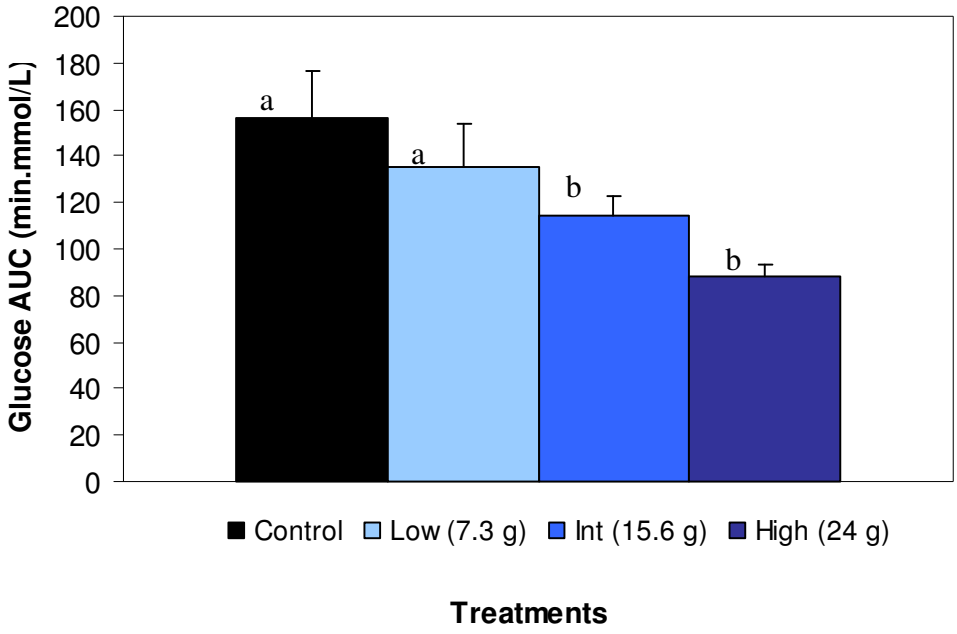
Statistical analysis was performed using NCSS 2000 (NCSS, Kaysville, UT) and SPSS release 16.0 (SPSS Inc., Chicago, IL). Incremental areas under the blood glucose response curves and appetite scores (iAUC) were calculated by applying the trapezoid rule and analyzed by one-way ANOVA using the Neuman-Keuls method to adjust for multiple comparisons. We performed Pearson correlations and conducted linear regression analysis to determine dose-response relationships for glucose and appetite iAUCs. Two-factor ANOVA was performed on blood glucose levels and appetite scores at each time point to test for a time x treatment interaction. When an interaction was statistically significant, a one-factor ANOVA using a GLM procedure was followed by Newman-Keuls post hoc test to identify mean differences among treatments at each time of measurement. Significance was set at $p < 0.05$.

4.4 Results

4.4.1 Postprandial Blood Glucose Responses

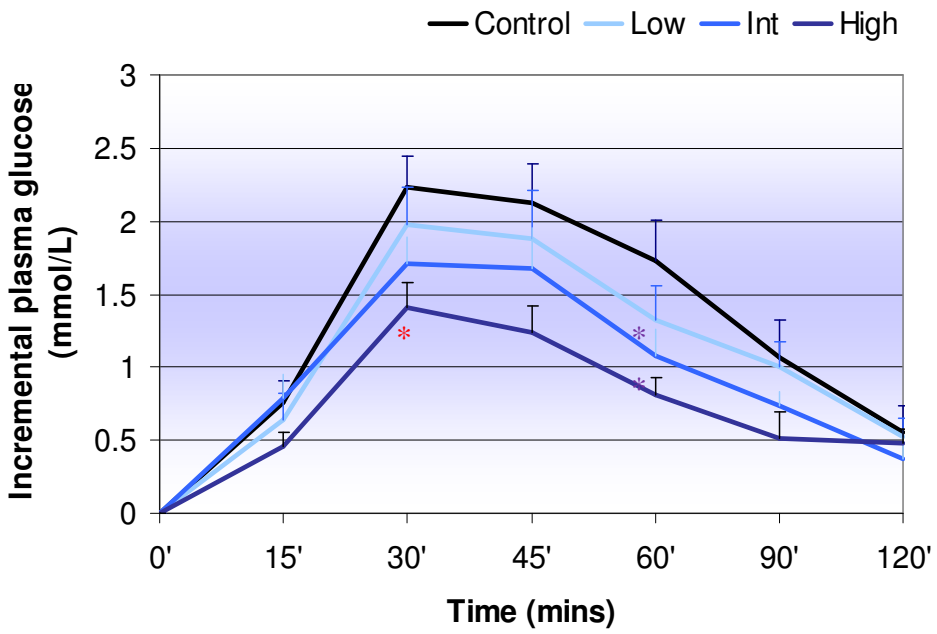
Blood glucose iAUC was negatively correlated with dose of Salba (r=-0.47 p=0.001) (Data not shown). Thus, approximately 22% of the variation in blood glucose could be accounted for by the dose of Salba. The mean glucose incremental AUC (± s.e.m.) was 156.6 ± 20, 135.6 ± 18.3, 114.5 ± 8.5 and 88.4 ± 5.7 min.mmol/L for control, low dose, intermediate dose and high dose breads, respectively. (Please see Table 4.1). Glucose iAUC was significantly reduced in both high (44%) and intermediate (25%) doses compared to control ($p = 0.002$). The low dose reduced iAUC by 13%, which did not reach significance. The incremental blood glucose value was significantly lower for the high dose compared to control at 30 mins (p=0.016) and for both high and intermediate doses at 60 mins postprandially (p=0.007).

Graph 4.1 The Effects of Salba on Blood Glucose iAUC (n=12)



p = 0.002; 1-way ANOVA; Newman-Keuls

Graph 4.2 The Effects of Salba on Incremental Blood Glucose (n=12)



* High < Control; p = 0.016
* High and Int < Control; p = 0.007
1-way ANOVA; Newman-Keuls

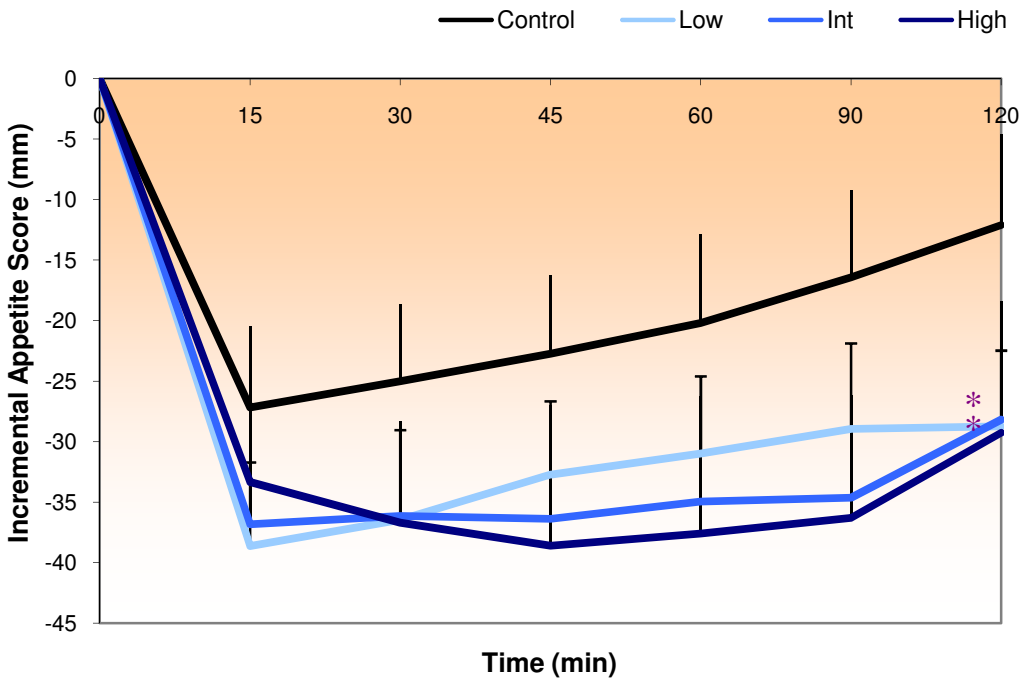
*Graph 4.2 The mean incremental glucose plasma glucose level at each time point. * indicates that High and Int are less than Control at 60 mins mins (p= 0.007) and * indicates that High is less than Control at 30 mins (p 0.016)*

4.4.2 Appetite Scores

Appetite scores were measured via four visual analog scales (VAS), and a combined score was calculated at fasting and for each of the following time points: 15, 30, 45, 60, 90 and 120 mins postprandially. Appetite score was inversely correlated with dose of Salba (r=-0.3, p=0.043). The satiety iAUC for low, intermediate and high doses were higher than control by 54.0%, 62.1% and 66.8% respectively (all NS, p= 0.108), with the mean satiety iAUC (± s.e.m.) of control, low, intermediate and high breads being -2400.2 ± 719.8, -3695.8 ± 950.1, -3890.3 934.5 ± 69.0 and -4003.6± 689.1 mm.min, respectively. Two-way ANOVA revealed a significant time by treatment interaction (p = 0.027). Analyzing incremental appetite scores over time, it was found that the appetite

scores for the high and intermediate doses at 90 mins (p = 0.044) and intermediate dose at 120 mins (p = 0.044) were significantly lower than control.

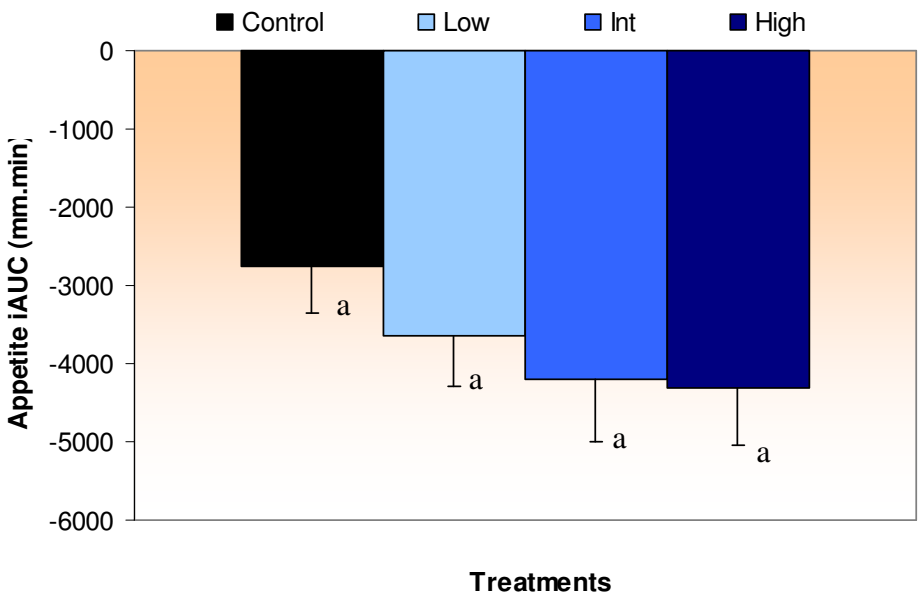
Graph 4.3 The Effects of Salba on Incremental Subjective Appetite (n=12)



*High and Int < Control; p= 0.044

*Graph 4.3 The mean incremental subjective appetite scores at each time point. * indicates that High and Int are less than Control at 120 mins (p= 0.044) and * indicates that Int is less than Control at 90 mins (p 0.045)*

Graph 4.4 The Effects of Salba on Satiety Score iAUC's (n=12)



p= 0.108; 1-way ANOVA

4.5 Discussion

Results showed that high (24g) and intermediate (15.6g) doses of Salba added to white bread containing 50g of available carbohydrate significantly reduced glucose iAUC by 44% and 24%, respectively. This is equivalent to an average iAUC reduction of 2% for one gram of Salba We cannot determine exactly which components of Salba reduced postprandial glycemia, as the breads differed in caloric value. In this case, we hypothesize that the added protein, fat and/or fiber from the Salba most likely contributed to the lowered blood glucose responses. The effects of Salba on postprandial glycemia may help explain the reduction in blood pressure, low-grade body inflammation and coagulation factors observed in Vuksan *et al.*'s long-term study. Postprandial hyperglycemia promotes oxidative stress, which in turn can cause or exacerbate risk factors for CVD such as those assessed. It follows, then, that reduction of postprandial glycemia may suppress the cascade of effects normally triggered by oxidative stress.

Appetite score was significantly decreased at 90 (high and intermediate doses) and 120 mins (intermediate dose) compared to control. However, there were no significant differences between the appetite iAUC's of all three Salba doses. Apparently the effectiveness of the appetite-suppressing

component does not act proportionally to its amount. The fact that the control and low dose breads elicited significantly higher glucose iAUC's than the other two breads but did not promote lower appetite appears to contradict the Glucostatic Theory at first glance. However, it is possible that since hypoglycemia was never reached (i.e. glucose level did not fall below baseline value) for either control or low dose bread, hunger signals were not triggered. On the other hand, we believe that appetite was marginally lower for intermediate and high dose breads despite eliciting lower blood glucose responses because of their greater fiber, protein, mineral and/or fat contents. Such components are proposed to promote certain satiety signals, such as the release of GLP-1.

As for the marginally greater effects on appetite for the intermediate dose bread compared to the high dose bread, we speculate that the high dose lowered postprandial glycemia to such a great degree that the blood glucose level was not high enough to elicit as strong satiety signals as the intermediate dose. Such a hypothesis would be in line with The Glucostatic Theory which states that satiety and blood glucose concentration are linearly correlated. As well, we theorize that the low dose and control breads did not elicit significantly decreased appetite despite causing greater blood glucose responses because they had lower fiber, protein, mineral and fat contents than the intermediate and high dose Salba breads.

We cannot determine specifically which aspect of the enriched breads lowered appetite, as the breads were not equicaloric. Thus, variables such as increased caloric value might have been responsible for the effect seen. Yet the changes in satiety were most likely not caused by the unequal caloric values of the preloads because the differences were too small. Referring to past research on energy intake and satiety, it is improbable that the differences of 29 and 59 kcal as used in our study would be sufficient to elicit a difference in appetite. For instance, Hulsof *et al.* found no difference in energy intake after two preloads differing by 700kcal. In the majority of studies that do find differences in subjective and/or objective satiety after experimental preloads, the preloads differed in calories by anywhere from 150 to 600 kcal. Nonetheless, future studies should address this issue by investigating the effects of equicaloric experimental meals on satiety.

In conclusion, this study demonstrates that the addition of Salba to a carbohydrate meal lowers the glycemic response and subjective appetite. Controlling these factors may help decrease the risk of certain diseases such as Type 2 diabetes and CVD. Future studies could assess the effectiveness of Salba in reducing postprandial glycemia in individuals with Type 2 diabetes. Furthermore, they should address the effectiveness of Salba to lower appetite by comparing equicaloric test meals and assessing subsequent food intake.

5 Study 2: The Effects of Escalating Doses of Whole vs. Ground Salvia hispanica L. (Salba) on Postprandial Glycemia and Subjective Appetite in Healthy Individuals

5.1 Abstract

Objectives: To assess if the effects of escalating doses of whole versus ground forms of the whole grain Salba on postprandial glycemia and subjective appetite compared to energy-matched white bread controls.

Methods Using an acute randomized controlled design, 20 healthy individuals (8M;12F; BMI 25.7 ± 2.4 kg/m^2) received on nine occasions either 7, 15 or 24 g of ground or whole Salba baked into white bread. There was one energy-matched control bread for each of the three doses of Salba. All meals contained 50 g of available carbohydrates. Fingerprick blood samples and ratings of satiety scores on a 100 mm visual analog scale were taken at fasting and 15, 30, 45, 60, 90 and 120 mins post-consumption.

Results. Blood glucose iAUC was negatively correlated to the dose of Salba of whole and ground forms combined (r=-0.17, p=0.035). There was a difference between iAUC's for the high doses only, with blood glucose responses to the two Salba breads significantly lower than that of the high dose control (p=0.02). Average percent reductions in appetite for whole and ground Salba breads combined relative to equicaloric controls were -5.3%, -9.5% and -14.8%, respectively.

Conclusions Salba's ability to lower postprandial glycemia is not fully due to its macronutrient proportion. As well, both ground and whole Salba are equally effective in lowering postprandial glycemia when added to white bread.

5.2 Introduction

The novel oil-rich whole grain *Salvia hispanica L.* (Salba) has been shown to reduce postprandial glycemia and subjective appetite when added to white bread. Such effects may help explain the lowered blood pressure, coagulation, low-grade body inflammation and adiposity seen in a previous long-term Salba studies. Postprandial glycemia has been shown to have predictive and diagnostic value for both Type 2 diabetes and cardiovascular disease. Postprandial hyperglycemia triggers harmful

effects on endothelial cell function, blood coagulation and body inflammation. Pharmacological and lifestyle interventions show that adequate control of 2-hour postprandial glycemic rises in individuals with diabetes or pre-diabetes can reverse the risk for and progression of cardiovascular disease and metabolic morbidities.

Recent data has shown that as little as 15g of Salba added to a 50g carbohydrate portion of white bread significantly lowers blood glucose iAUC and subjective appetite in the 2 hours postprandially. Since this represents the addition of only 55kcal, the incorporation of Salba to high glycemic index meals may prove to be a simple and effective measure to prevent postprandial hyperglycemia and the resulting detrimental atherosclerotic changes. As well, past research suggests that the addition of only 55kcal of Salba to white bread significantly decreases subjective appetite, which in turn could reduce overconsumption. However, equicaloric test meals were not used. Here, we investigated the effects of calorie-, protein- and fat-matched white bread controls versus Salba-enriched white breads to elucidate possible factors that contributed to the previous findings, especially those concerning appetite.

In addition, Salba is commercially available in both whole grain and ground forms, yet any differences in effects on postprandial glycemia and appetite have not been assessed. Few studies have investigated the differences of oily grains or seeds in ground and whole form. In this study we compared the effectiveness of both forms in reducing glycemic response and subjective appetite.

5.3 Method

5.3.1 Participants

A total of 20 healthy individuals (8M; 12F; Age: 39.4 ± 3.4 years BMI 25.7 ± 2.4 kg/m^2) participated in the study. All participants were healthy, between 18-65 years old and clinically euthyroid with normal hepatic and renal function. Those who were pregnant, suffered from gastrointestinal or metabolic diseases or regularly ingested fiber supplements were excluded. All participants gave written informed consent, and the study was approved by the St. Michael's Hospital Research Ethics Board.

5.3.2 Treatments

The test meals consisted of servings of bread (served with 250 millilitres of water) enriched with 7, 15 or 24 g of ground or whole Salba. The lowest dose, 7.3g, contains the American Heart Association's daily minimum recommended intake of omega-3 fatty acids. The three calorie-, protein- and fat-

matched controls consisted of white bread baked with egg whites and margarine to match the three doses of Salba. All 9 experimental breads contained 50g available carbohydrate and were prepared on-site with a Black & Decker® All-In-One Pro™ Breadmaker (Towson, MD, USA)

Table 5.1 Nutritional Facts of Salba-enriched Breads

	Low Dose	Intermediate Dose	High Dose
Serving Size	110g	117g	126g
Calories	260 kcal	292 kcal	324 kcal
Fat	3.4g	6.0g	9.0g
Carbohydrate	55.0g	57.9g	60.7g
Fiber	5.0g	7.9g	10.7g
Available Carbohydrate	50.0g	50.0g	50.0g
Protein	3.4g	6.0g	9.0g

Table 5.2 Nutritional Facts of Control Breads

	Low Dose	Intermediate Dose	High Dose
Serving Size	103g	113g	110g
Calories	260 kcal	292 kcal	325 kcal
Fat	3.4g	6.0g	9.0g
Carbohydrate	55.0g	57.9g	60.7g
Fiber	5.0g	7.9g	10.7g
Available Carbohydrate	50.0g	50.0g	50.0g
Protein	3.4g	6.0g	9.0g

5.3.3 Experimental Design

The study consisted of a double-blind, placebo-controlled, randomized crossover design in which subjects underwent nine 2.5-hour sessions separated by a washout period of at least 48 hours. Subjects visited the Risk Factor Modification Centre in the morning after having fasted for 10-12 hours overnight and engaging in normal eating and exercise habits the preceding day. They had an initial

finger prick blood sample taken and completed a subjective appetite questionnaire in the form of a 100mm visual analog scale (VAS). The experimental meal was then served and subjects were required to finish the meal within 15 mins. Finger prick blood samples were taken at 15, 30, 45, 60, 90 and 120 mins postprandially. Subjects also completed the appetite questions at these times. For the duration of the study session, subjects remained at the clinic and were instructed not to eat or drink and to keep physical activity to a minimum.

5.3.4 Blood Glucose Analysis

Capillary blood samples were obtained using sterile single-use lancets. Two to three drops of capillary blood were collected in plastic flat-bottomed 5ml tubes with a push cap containing a small amount of sodium fluoride and potassium oxalate as an anticoagulant and preservative. The blood samples were placed in a -20ºC freezer for a maximum of 3 days until the analysis of whole blood glucose. Capillary blood glucose was measured by the glucose oxidase method using a YSI 2300 STAT Plus Glucose & Lactate Analyzer™ (Yellow Springs Instruments, Yellow Springs, OH, USA).

5.3.5 Measurement of Appetite

Four unipolar visual analogue scales were used for each appetite assessment. For each of the four questions, subjects indicated their response by drawing a vertical line along a 100 mm horizontal line that was anchored by two statements. The questions were “How strong if your desire to eat?”, “How hungry do you feel?”, “How full do you feel?” and “How much do you think you could eat now?” Subjects’ ratings were converted to numerical values by measuring the distance between the left anchor and their drawing. A combined appetite score for each appetite assessment was computed with the following formula:

$$[Q1 + Q2 + Q4 + (100 - Q3)] / 4 \quad [141]$$

5.3.6 Study Variables

The primary variables for this study were the mean incremental AUC’s for blood glucose and the mean incremental change from baseline in blood glucose at 15, 30, 45, 60, 90 and 120 min postprandially.

The secondary variables were the mean iAUC's of appetite scores and the incremental change in appetite from baseline at the same time points.

5.3.7 Statistical Analysis

Statistical analysis was performed using NCSS 2000 (NCSS, Kaysville, UT) and SPSS release 16.0 (SPSS Inc., Chicago, IL). Incremental areas under the blood glucose response curves and appetite scores (iAUC) were calculated by applying the trapezoid rule and analyzed by one-way ANOVA using the Neuman-Keuls method to adjust for multiple comparisons. We performed Pearson correlations and conducted linear regression analysis to determine dose-response relationships for glucose and appetite iAUCs. Two-factor ANOVA was performed on blood glucose levels and appetite scores at each time point to test for a time x treatment interaction. When an interaction was statistically significant, a one-factor ANOVA using a GLM procedure was followed by Newman-Keuls post hoc test to identify mean differences among treatments at each time of measurement. Significance was set at $p < 0.05$.

5.4 Results

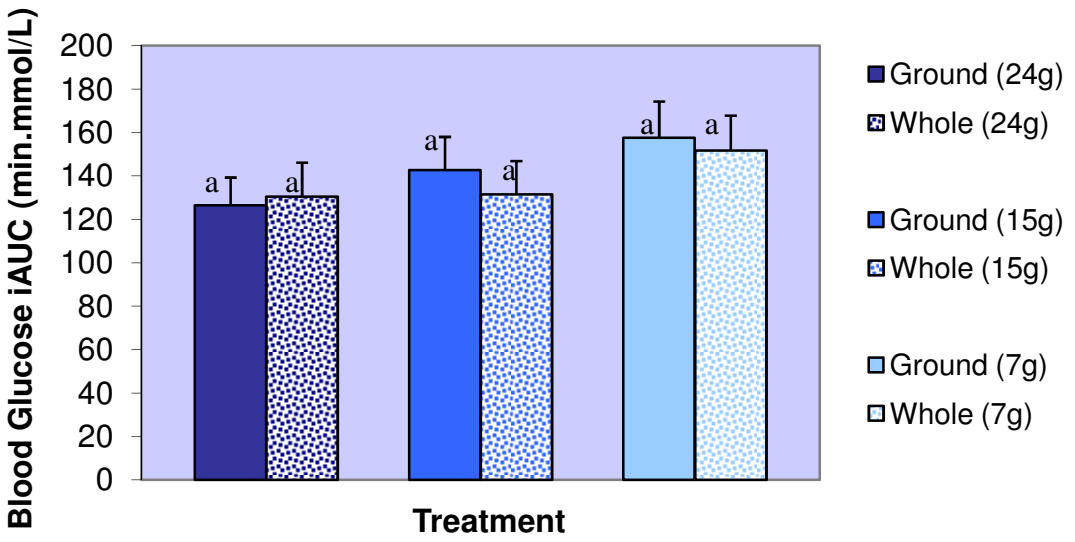
5.4.1 Postprandial Blood Glucose Responses

The means of glucose iAUC's for all nine experimental breads are shown in Table 5.3 and Graph 5.1. Glucose iAUC was negatively correlated to dose of Salba of ground and whole forms combined (r=0.17, p=0.035). There was no significant difference found between the iAUC's of all three calorie-matched control breads (HC = 154.6 ±13.0, IC = 154.3 ±17.1 and LC = 160.8 ± 16.8 min.mmol/L, p = 0.87). The iAUC's for the HG, IG and LG were 126.5 ± 12.7, 142.7 ± 15.2 and 157.9±16.7 mmol.min/L, respectively. The iAUC's for the HW, IW and LW were 130.5 ± 15.6, 139.2 ± 15.4 and 151.6±16.1 mmol.min/L, respectively.

Table 5.3 Glucose iAUC (min.mmol/L) values (± sem) for all Experimental Breads

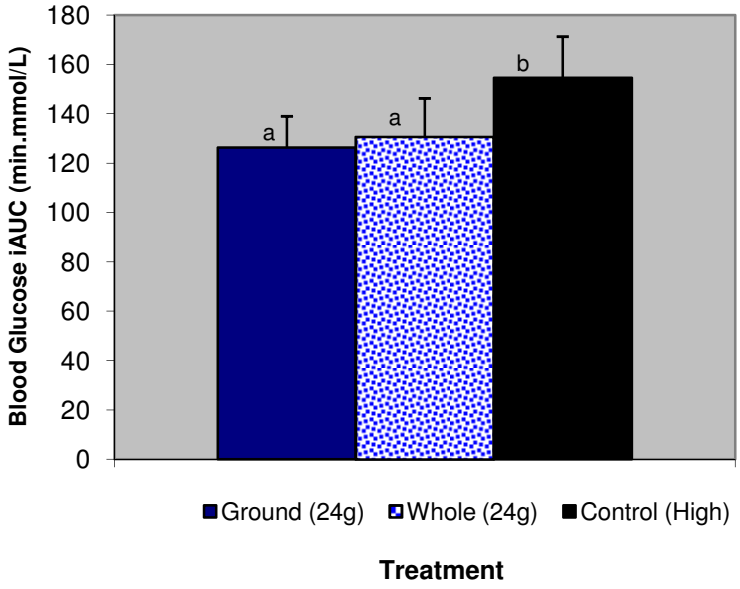
	Ground	Whole	Control
High	126.5 ± 12.7 [a]	130.5 ± 15.6 [a]	154.6 ±13.0 [b]
Intermediate	142.7 ± 15.2	139.2 ± 15.4	154.3 ±17.1
Low	157.5±16.7	151.6±16.1	160.8 ± 16.8

Graph 5.1 The Effects of Salba on the Blood Glucose iAUC of all Salba Breads (n=20)



When comparing the breads within each dose group, it was found that there was a difference between the AUC's of the three high dose breads only. One-way ANOVA of the iAUC's of the high dose breads indicated a significant difference between both HG and HW with respect to HC ($p = 0.021$). Analysis by two-factor ANOVA identified a time by treatment interaction within the high dose breads ($p=0.024$). However, 1-way ANOVA revealed no significant differences in high dose incremental glucose values at any of the time points.

Graph 5.2 The Effects of the High Dose Breads on Blood Glucose iAUC (n=20)



p=0.02; 1-way ANOVA, Newman-Keuls

A mild dose-response effect was seen within the ground breads, with the iAUC of HG significantly lower than LG (p=0.033) (data not shown). In regards to the whole breads, IW tended to be significantly lower than LW but did not reach significance (p= 0.06). Analysis of incremental glucose values also indicated a dose-response effect. Within the 3 doses of ground breads, the HG and IG were significantly less than LG at 60 mins postprandially (data not shown).

5.4.2 Appetite Scores

Appetite scores were measured via four visual analog scales (VAS), and a mean combined score was calculated for each of the following time points: fasting, 15, 30, 45, 60, 90 and 120 mins postprandial. No correlation was found between appetite score and dose of Salba (p=0.29). The appetite score iAUC's were not significantly different within each dose (and thus within equicaloric breads), as p=0.29, 0.27 and 0.48 for high, intermediate and low doses, respectively. See Table 5.5 for iAUC values and Table 5.6 for percent reductions in appetite of Salba breads.

Table 5.4 Appetite iAUC's of Experimental Breads

	High Dose	Intermediate Dose	Low Dose
Ground	-4258 ± 689.9 mm.min	-4438 ± 612.6 mm.min	-4315 ± 599.6 mm.min
Whole	-5064 ± 669.7 mm.min	-4662 ± 594.8 mm.min	-4274 ± 666.3 mm.min
Control	-4420 ± 691.2 mm.min	-4049 ± 625.9 mm.min	-3827 ± 675.0 mm.min

Table 5.5 Percent Reductions of Appetite iAUC's of Salba Breads with Respect to Calorie-, protein- and fat-matched Control Breads

	High Dose	Intermediate Dose	Low Dose
Ground	4.0%	-4.0%	-12.8%
Whole	-14.6%	-15.0 %	-16.8 %

However, two-factor ANOVA of the appetite scores of the high dose breads revealed a significant time x treatment interaction ($p = 0.032$). Intermediate whole bread had a lower mean appetite score than intermediate control bread at 120 mins postprandially, yet this did not reach significance ($p = 0.056$)

5.5 Discussion

Results indicated that Salba is most effective at reducing postprandial glycemia at the highest dose, which was 24g per 50g available carbohydrate. Perhaps the differences in nutrient composition between the other doses of test and control breads were too small to be seen with this sample size. Nonetheless, a significant linear correlation between Salba dose and postprandial glycemia was found (r=0.17, p=0.035).

The proportion of fat and protein in Salba cannot completely account for the iAUC reduction for both HW and HG compared to HC as they were constant across the treatments, yet postprandial glycemia differed. Put differently, adding Salba to white bread does not reduce postprandial glycemia solely because of the added fat and protein. It can thus be concluded that there is some aspect of the grain

that is partly responsible for its ability to lower postprandial glycemia other than its protein and fat contents. It is possible that the specific types of fat and protein in Salba may act synergistically to lower blood glucose. As well, the amount of fiber and/or the proportion of soluble vs. insoluble fiber are possible contributing factors, as is the degree of saturation of the fat. Future acute studies could employ controls that contain the same type of protein, same type of fat and/or an equal amount of fiber as Salba to adjust for these factors.

However, past research has demonstrated that the differences in energy from fat and protein in our experimental breads were insufficient to elicit a difference in postprandial glycemic response. Studies investigating the addition of fat to a carbohydrate meal use a substantially greater amount of fat than the present study. For instance, Gatti *et al.* added 35g of fat to 75g carbohydrate to produce 70-80% reductions in glucose iAUC, while Collier *et al.* reported reductions of 10-20% when 37.5g of fat were added to 75g of carbohydrate [120, 127]. Owen and Wolever added 0, 5, 10, 20 and 40g of fat in the form of non-hydrogenated margarine and found that although the lowest amounts of fat lowered postprandial glycemia to a proportionally larger degree, only the highest amount of fat elicited a significant decrease in iAUC [118].

The studies investigating the addition of protein to carbohydrate meals show that protein does not elicit a consistent effect on postprandial glycemia. Some studies conclude that added protein lowers the glycemic response, while others show no difference [134]. One even reported an increase in glucose AUC when protein was added to a carbohydrate and fat meal [135]. Yet, when protein is ingested alone it has no or very little effect on blood glucose in people with or without Type 2 diabetes [136, 137]. Thus, it is very unlikely that the observed differences in iAUC in our study were due to the small variations in calories from protein and fat. Again, we speculate that an aspect of Salba such as its high soluble fiber content was responsible for lowering postprandial glycemia.

The reductions in postprandial glycemia were not as pronounced as the first study. We speculate that the addition of fat and protein to the control meals in the present study reduced the blood glucose response compared to white bread control to such an extent that the differences in effects of Salba and control breads were noticeably smaller. Therefore, the proportion of fat and/or protein did have an effect on postprandial glycemia; however, it was not completely responsible for the changes in blood glucose response.

To our knowledge, there have been very few studies examining the effects of oily foods such as flax and sesame seed in ground and whole forms on postprandial glycemia. One study assessed postprandial glycemia elicited by white bread enriched with flax. Only ground flax was used, however [189]. Most nutrition experts recommend the consumption of ground flax as opposed to whole flax to maximize the

its health benefits [190]. They contend that with its tough husk, whole flax can pass through the body undigested, and thus fewer nutrients are absorbed. Salba, on the other hand, has a very permeable husk which in theory would allow digestive enzymes easier access to its nutrients. There has also been research determining the effects of different food forms of legumes on postprandial glycemia; however, given their relatively high starch contents, we feel that this research is not applicable to Salba [191]. Regarding research on other whole grains and postprandial glycemia, the typical effects of whole grain particle size were not seen with Salba. Extensive research demonstrates that a grain's ability to lower postprandial glycemia is positively correlated with increasing particle size [192]. Researchers theorize that since bigger particles have a smaller surface-to-volume ratio, their contents are less accessible to digestive enzymes. As such, the contents of bigger particles are released and digested more slowly, leading to a flattened and/or prolonged glucose response [193].

However, we expected the opposite effect with Salba, and we attribute this to the grain's extremely low carbohydrate content and high content of components capable of reducing postprandial glycemia. Firstly, carbohydrate is the primary macronutrient responsible for raising blood glucose, but there is a negligible amount of carbohydrate in the grain. In addition, the contents of the intact Salba grain are mainly fat, protein and fiber – all components reported to lower glycemic responses. Therefore adding Salba to a carbohydrate meal contributes only an inconsequential amount of carbohydrate while providing components known to lower glycemia. When whole Salba is consumed, we would expect some interaction between digestive enzymes and its contents because the grain's soft husk is easily broken, which results in a modest reduction of blood glucose. In contrast, there would be a much greater surface area for enzyme-food interactions with ground Salba, allowing the contents to interact with digestive enzymes more easily. Thus, it would be expected that ground Salba would lower postprandial glycemia to a greater extent than whole Salba.

With regards to appetite, it appears that Salba has a very modest ability to lower subjective appetite. The iAUC's for appetite scores did not differ within each dose. However, there is evidence to suggest that whole Salba bread is more satiating than control bread. When the IG, IW and IC were analyzed together by 2-way ANOVA, it was found that IW tended to have a lower mean appetite score than IC at 120 mins postprandially ($p = 0.056$). It is possible that the amount of calories from Salba and differences in calories tested in this thesis were too little to see greater effects on satiety. Future studies could employ larger differences in Salba to determine the extent of its appetite-lowering abilities.

One possible explanation for why whole Salba elicited lower appetite than control and why the ground form did not is the difference in effort during its consumption. The increased "crunchiness" of the whole Salba requires more force during mastication, which in turn may create greater feelings of

satiety. Haber *et al*, 1977 noted that apple pieces reduced hunger to a greater extent than an equicaloric amount of apple purée and suggested that the disruption of fiber increases subjective appetite, partly due to the less mastication required [194]. In contrast, a high fiber food requires more mastication, which results in greater satiety. Chewing solid or viscous food as has been reported to increase satiety signals and hormones compared to simply swallowing liquid, which requires no chewing [195].

6 General Discussion and Conclusion

6.1 Summary

This thesis sought to investigate the effectiveness of the novel oily whole grain *Salvia hispanica L.* (Salba) on postprandial glycemia and subjective appetite via two acute studies. Study 1 evaluated the effects of three doses of ground Salba added to white bread on the postprandial glycemic response and subjective appetite. Results showed that Salba reduced both measures in a dose-dependent manner in healthy individuals. Study 2 examined the effects of whole versus ground Salba on postprandial glycemia and subjective appetite. We demonstrated that the ground and whole forms are approximately equal in effectiveness. As well, Study 2 sought to determine if Salba's protein and fat contents are responsible for its ability to lower postprandial glycemia and appetite. We thus employed equicaloric protein- and fat-matched white bread controls by adding margarine and egg white to white bread.

6.2 The Effects of Salba on Postprandial Glycemia

The addition of 7, 15 and 24g of Salba to white bread containing 50g available carbohydrate lowered postprandial glycemia in a dose-dependent fashion. Linear regression analysis showed a significant dose-response decrease in blood glucose incremental area under the curve with increasing doses of Salba (iAUC). In other words, blood glucose iAUC was negatively correlated with dose of Salba (r=-0.47). The amount of Salba therefore accounts for approximately 22% of the variation in postprandial glycemia. For the ground Salba-enriched breads, glucose iAUC reductions of 44%, 25% and 13% were seen for the high, intermediate and low doses compared to white bread, respectively. Only the reductions for the high and intermediate doses were significant. The incremental blood glucose level was significantly lower for the high dose compared to control at 30 mins and for both high and intermediate doses at 60 mins postprandially.

For Study 2, each dose category was analyzed separately. Glucose iAUC was negatively correlated to dose of Salba of ground and whole forms combined (r=0.17). It was found that there was a significant difference between iAUC's for the three high dose breads only. Incremental blood glucose levels to HG and HW were significantly lower than that of HC (p=0.024). Further analysis by 1-way ANOVA yielded no significant differences for incremental glucose values within each dose category.

Data from Study 2 suggest that the ground form of Salba is marginally more effective at reducing the postprandial glycemic response than the whole form. Although 1-way ANOVA of glucose values indicated no significant differences within each dose, 2-way ANOVA revealed that the glucose values for the HG and HC differed significantly, with that of the former being lower. We cannot dismiss the possibility that these results occurred due to random variation, and thus suggest future studies with greater doses of ground and whole Salba.

6.3 The Effects of Salba on Subjective Appetite

The results of our studies suggest that Salba has a very modest ability to lower subjective appetite. In Study 1, linear regression analysis of appetite demonstrated a significant dose-response decrease in appetite iAUC with increasing doses of Salba (r=-0.3, p=0.043). Dose of Salba can therefore account for approximately 9% of the variation in appetite iAUC. The appetite score iAUC for low, intermediate and high doses were lower than control by 54.0%, 62.1% and 66.8%, respectively. Although these reductions occurred in a dose-dependent manner, no significant differences in satiety score iAUC's were found between high, intermediate and low Salba breads. Appetite scores were significantly higher for the high dose at 90 mins and for the intermediate dose at 90 and 120 mins.

These results were unexpected and appear to be counterintuitive, as the intermediate dose contains fewer calories than does the high dose. It is therefore possible that there exists an optimal amount of Salba to add to a carbohydrate meal in order to achieve maximum satiety. More likely however, is that there is an optimal ratio of calories from Salba to total calories from the entire carbohydrate meal to elicit the greatest reduction in appetite. Both scenarios would require producing the optimal postprandial glycemic response for lowering appetite: a rise in blood glucose sufficiently low and prolonged to prevent hyperglycemia, while maintaining a concentration high enough to trigger satiety signals.

Results from Study 2 also provide evidence for Salba's modest ability to decrease appetite. Although appetite score iAUC's were not significantly different within each dose (and thus within equicaloric breads), intermediate whole bread tended to have a lower appetite score than intermediate control bread at 120 mins (p = 0.056). Unlike Study 1, however, no linear correlation was found between appetite score and dose of Salba.

It is noteworthy that such a small addition of calories in the form of Salba reduced subjective appetite. As mentioned previously, the majority of appetite studies require differences of more than 100kcal for an effect to be apparent. The results of the intermediate breads suggest that Salba could be more satiating than the same macronutrient proportion and amount of calories as another food source (in this study, white bread baked with egg white and margarine).

6.4 Relevance of Findings

Epidemiological evidence suggests that postprandial glycemic rises are an independent and modifiable predictor of Type 2 diabetes and cardiovascular disease. The whole grain Salba has the ability to lower postprandial glycemia when incorporated into a carbohydrate-rich meal, which represents a possible method in reducing risk factors for such diseases. Salba may even prove to be sufficiently effective in some cases to replace medications designed to lower postprandial glycemia, such as acarbose. Our finding that one gram of Salba decreased glucose iAUC by approximately 2% could be useful in determining the amounts required per carbohydrate-rich meal.

Our data suggest that consumption of Salba may also target another possible risk factor for the development of Type 2 Diabetes and cardiovascular disease: obesity. Salba has a fairly small ability to lower subjective appetite, which we hypothesize would lead to less energy consumption. At 120 mins postprandially, the appetite score of the intermediate whole Salba bread was tended to be lower than intermediate control. When added to a portion of white bread, whole Salba may increase satiety more than an equal amount of calories from another source. Thus, adding Salba to one's diet could be a valuable and simple dietary modification for individuals with excess bodyweight. Most people attempting to lose weight consume calorie-restricted diets. Unfortunately, many of these diets lack one or more essential macro- or micronutrients, especially unsaturated fats, minerals and proteins. Salba would be an ideal complementary food for a calorie-restricted weight loss diet as its nutrient-dense composition would help alleviate any deficiencies.

Additionally, this thesis has clarified the effects of a range of Salba doses and the efficacy of both ground and whole forms. These results could be applied to the manufacturing of baked products with oily seeds such as flax and sunflower.

6.5 Limitations

The findings of this project should be considered in light of the limitations pertaining to the study concept. Equicaloric breads were not used in Study 1, rendering it difficult to compare the subjective appetite results from each experimental bread. This issue was addressed in Study 2 with the use of a calorie-matched white bread control for each dose of Salba. However, the addition of fat and protein to the white bread controls caused the blood glucose responses to be lower than white bread alone since both macronutrients have been shown to reduce postprandial glycemia. In doing so, we were only examining the ability of the fiber and mineral contents of the Salba to lower postprandial glycemia. This may thus account for the smaller decreases in postprandial glycemia in Study 2 compared to Study 1.

Additionally, a slight difference in procedure between Studies 1 and 2 may also have contributed to the fact that the decrease in blood glucose response was less drastic in the latter. For Study 1, bread baked less than 48 hours previously was given to participants. In contrast, bread was frozen and thawed in Study 2, due to temporal and logistical restraints. Research has suggested than freezing and thawing fiber-rich foods can destruct the physical structures of the fibers, resulting in diminished effects on postprandial glycemia [196].

With respect to both studies, appetite was measured via visual analog scales, and thus represents solely subjective feelings. Past research has shown that desire and intentions to eat do not consistently correlate with actual food intake [141, 177, 197]. Thus, our research would have benefited from measuring subsequent food intake via an *ad libitum* meal or 24-hour food diaries. Furthermore, we could not adjust for differences in density of the experimental breads. The breads enriched with ground Salba had a denser texture than both the whole and control breads. The calorie- and macronutrient-matched control breads had a significantly fluffier texture, which we attribute to the addition of egg whites. Burton and Lightowler reported that denser breads elicited greater feelings of satiety and lower peak glucose levels than less dense breads [198]. Yet research by Rolls *et al.* has demonstrated that food volume is negatively correlated to subsequent food intake [167, 168]. As these findings are contradictory, it is difficult to suggest how the different bread densities in our study affected satiety. As well, the density of a food is by nature known by the individual consuming it; thus, equalizing densities may not be relevant.

Lastly, it was impossible to keep the whole Salba breads completely double-blinded. Participants can obviously perceive differences between the breads with whole Salba versus those without, and it is

possible that the perception of whole Salba grains in the bread may influence subjective appetite. It would be difficult, however, to distinguish between low, intermediate and high dose whole Salba breads. As well, the knowledge of the whole Salba bread's texture is a legitimate characteristic that may affect appetite and is therefore simply another property of whole Salba that promotes appetite reduction.

6.6 Future Research

This thesis paves the way for many future avenues of research. Further investigations should address the limitations of this project and the research questions that arose from it. The effects of Salba on appetite could be more thoroughly examined with acute studies measuring objective satiety with an *ad libitum* meal and/or 24-hr food diaries. In addition, acute studies measuring key regulatory gut hormones such as CCK and GLP-1 would be valuable in determining the metabolic effects of this grain and elucidating the mechanisms by which it increases satiety and decreases food intake.

Our research demonstrated that the amount of protein and fat in Salba-enriched bread is not an important factor in reducing postprandial glycemia and appetite. It may be Salba's physical structure and/or its fiber, antioxidant or unsaturated fat contents, among others. Future studies should focus on matching the content of such components in control meals to isolate which nutrients are responsible for the health benefits seen in this study.

The results from our research dovetail with those of Vuksan *et al.*'s research as they both demonstrated Salba's potential to reduce possible CVD risk factors. Further studies should be undertaken to determine if these results are replicable, and if so, examine other criteria such as the daily amount of Salba required and effects on additional risk factors. Our research provides another mechanism by which Salba can promote weight loss; namely, by lowering appetite. As such, randomized, parallel studies could examine both of these effects by measuring anthropometrics and food intake to explore this issue in further detail.

Furthermore, Salba may directly affect body composition by decreasing fat mass. Research by both Chicco and Vertommen suggest that consumption of Salba has the potential to lower adiposity. Chicco *et al.* studied the effects of a diet including *Salvia hispanica L.* in rats after a 5-month high-sucrose diet [5]. They found that visceral adiposity (epididymal and retroperitoneal fat) was significantly decreased relative to body weight for the rats on the *Salvia hispanica L.* diet versus rats on a maize diet. In a fluidity study with healthy individuals, Vertommen *et al.* demonstrated a significant decrease in waist circumference while maintaining body weight after merely 50g Salba per day for one month [20]. Thus, given the significant evidence showing that Salba may specifically target the loss of fat mass, future long-term studies analyzing body composition are warranted.

This thesis has helped determine a range of optimal amounts of Salba to consume and the efficacy of both ground and whole forms. Further research could be performed with Salba in ground and whole forms in other solid foods and beverages to investigate whether food forms have similar health effects

in different food matrices. Salba is sometimes consumed as a drink in South America and some North American companies have expressed interest in developing a beverage.

In conclusion, further studies are needed to elucidate the mechanisms by which Salba reduces postprandial glycemia and appetite, and if doing so reduces risk factors for Type 2 diabetes and CVD. Metabolic studies will further our understanding of the effects of Salba on risk factors such as glucose and insulin response, blood lipids, clotting factors and low-grade body inflammation. As well, examining the effects of Salba on satiety and body weight regulation is of particular interest as a preventative measure in these diseases.

6.7 Conclusion

The oily whole grain Salba has the ability to significantly reduce postprandial glycemia when added to a carbohydrate meal. Salba-enriched white bread also elicits lower, non-significant ratings of subjective appetite than equicaloric protein- and fat-matched white bread. Prevention of both postprandial hyperglycemia and overconsumption may reduce the risk of diseases such as Type 2 diabetes and CVD in the long-term. Incorporating Salba in the diet could be both an excellent method of attaining the recommended daily servings of fiber and omega-3 fatty acids and of obtaining essential nutrients lacking from calorie-reduced diets.

7 References

1. Ayerza, R. and W. Coates, *Chia: Rediscovering a Forgotten Crop of the Aztecs*. 2005, Arizona, USA: University of Arizona Press.

2. Riccardi, G., B. Capaldo, and O. Vaccaro, *Functional foods in the management of obesity and type 2 diabetes.* Current Opinion in Clinical Nutrition & Metabolic Care, 2005. **8**(6): p. 630-5.

3. Anonymous, *Bibliography. Current world literature. Functional foods.* Current Opinion in Clinical Nutrition & Metabolic Care, 2007. **10**(6): p. 778-90.

4. Vuksan, V., et al., *Supplementation of conventional therapy with the novel grain Salba (Salvia hispanica L.) improves major and emerging cardiovascular risk factors in type 2 diabetes: results of a randomized controlled trial.* Diabetes Care, 2007. **30**(11): p. 2804-10.

5. Chicco, A., et al., *Dietary chia seed (Salvia hispanica L.) rich in a-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats.* British Journal of Nutrition, 2008: p. 1-10.

6. Vuksan, V., *Salba vs. Chia*. 2006: Toronto.

7. Anderson, J.W., et al., *Whole grain foods and heart disease risk.* Journal of the American College of Nutrition, 2000. **19**(3 Suppl): p. 291S-299S.

8. Anderson, J.W., *Whole grains protect against atherosclerotic cardiovascular disease.* Proceedings of the Nutrition Society, 2003. **62**(1): p. 135-42.

9. de Munter, J.S., et al., *Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review.* PLoS Medicine / Public Library of Science, 2007. **4**(8): p. e261.

10. Jacobs, D.R., Jr, et al., *Fiber from Whole Grains, but not Refined Grains, Is Inversely Associated with All-Cause Mortality in Older Women: The Iowa Women's Health Study.* Journal of the American College of Nutrition J Am Coll Nutr, 2000. **19**(90003): p. 326S-330.

11. Koh-Banerjee, P. and E.B. Rimm, *Whole grain consumption and weight gain: a review of the epidemiological evidence, potential mechanisms and opportunities for future research.* Proceedings of the Nutrition Society, 2003. **62**(1): p. 25-9.

12. Pereira A, J.D., Slattery M, Ruth K, Van Horn L, Hilner J & Kushi L, *The association of whole grain intake and fasting insulin in a biracial cohort of young adults: the CARDIA study.* CVD Prevention, 1998. **1**: p. 231-242.

13. Pereira, M.A., et al., *Effect of whole grains on insulin sensitivity in overweight hyperinsulinemic adults.* American Journal of Clinical Nutrition, 2002. **75**(5): p. 848-55.

14. Steffen, L.M., et al., *Whole grain intake is associated with lower body mass and greater insulin sensitivity among adolescents.* American Journal of Epidemiology, 2003. **158**(3): p. 243-50.

15. Jacobs, D.R., et al., *Fiber from whole grains, but not refined grains, is inversely associated with all-cause mortality in older women: the Iowa women's health study.* Journal of the American College of Nutrition, 2000. **19**(3 Suppl): p. 326S-330S.

16. *Salba: Nature's Most Powerful Whole Food*. 2007, SourceSalba.

17. *Nutrient Composition of Salba Seeds*. 2001, Dr. David Jenkins Lab [Report, Personnal Communication], University of Toronto: Toronto.

18. Wu, X., et al., *Lipophilic and hydrophilic antioxidant capacities of common foods in the United States.* Journal of Agricultural & Food Chemistry, 2004. **52**(12): p. 4026-37.

19. Beck, L., *It's time to cut the fat and go for the whole grain*, in *Globe and Mail*. 2008: Toronto.

20. Vertommen, J., et al. *Efficacy and Safety of 1 Month Supplementation of SALBA (Salvia Hispanica Alba)Grain to Diet of Normal Adults on Body Parameters, Blood Pressure, Serum Lipids,Minerals Status and Haematological Parameters. Results of a Pilot Study*. in *24th International Symposium on Diabetes and Nutrition*. 2006. Salerno, Italy.

21. *USDA Nutrient Data Laboratory*. 2008, United States Department of Agriculture.

22. NutritionData.com, *Nutrition Data*, Condé Nast Publications.

23. Faulkner, H., *Nutritional Labeling Analysis (Salba seeds).* 2001, [Report, Personal Communication] University of Guelph: Guelph.

24. *Fatty Acid Profile of Salba Seeds*. 2001, Dr. Steven Cunnane's Lab, University of Toronto: Toronto.

25. *Fiber Content of Salba Seeds*. 2001, [Report, Personal Communication] Maxxan Analytics.

26. *Welcome to AACCNet*. 2008, American Association of Cereal Chemists.

27. Sungsoo Cho, S., *Handbook of Dietary Fiber*. 2001, Maryland: CRC Press. 894.

28. Schneeman, B. and T. J, *Modern Nutrition in Health and Disease*. 8 ed. ed, ed. M. Shike, M. Shills, and J. Olsen. 1994, Philadelphia: Lea & Febiger.

29. *Physiological effects and health consequences of dietary fiber*, ed. S.M. Pilch, Bethesda, Md. (USA): Life Sciences Research Office, Federation of American Societies for Experimental Biology.

30. *Dietary Reference Intakes Proposed Definition of Dietary Fiber: A Report of the Panel on the Definition of Dietary Fibre and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes Food and Nutrition Board.* 2001, Institute of Medicine: Washington, D.C.

31. Vuksan, V., et al., *Konjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes. A randomized controlled metabolic trial.* Diabetes Care, 1999. **22**(6): p. 913-9.

32. Jenkins, D.J., et al., *Improved glucose tolerance four hours after taking guar with glucose.* Diabetologia, 1980. **19**(1): p. 21-4.

33. Anderson, J.W., *Dietary fiber, lipids and atherosclerosis.* American Journal of Cardiology, 1987. **60**(12): p. 17G-22G.

34. Ausman, L.M., *Fiber and colon cancer: does the current evidence justify a preventive policy?* Nutrition Reviews, 1993. **51**(2): p. 57-63.

35. Lands, W., *Biochemistry and physiology of n-3 fatty acids.* FASEB J., 1992. **6**(8): p. 2530-2536.

36. Lunn, J. and H.E. Theobald, *Briefing Paper: The health effects of dietary unsaturated fatty acids*. 2006, Brisith Nutrition Foundation: London. p. 178-224.

37. Stark, A.H., M.A. Crawford, and R. Reifen, *Update on alpha-linolenic acid.* Nutrition Reviews, 2008. **66**(6): p. 326-332.

38. Simopoulos, A., A. Leaf, and N. Salem Jr. *Statement on the essentiality of and recommended dietary intakes for n-6 and n-3 fatty acids*. 2000. The Center for Genetics, Nutrition and Health.

39. Bazinet, R.P., H. Douglas, and S.C. Cunnane, *Whole-body utilization of n-3 PUFA in n-6 PUFA-deficient rats.* Lipids, 2003. **38**(2): p. 187-9.

40. DeFillipis, A. and L. Sperling, *Understanding omega-3's.* American Heart Journal, 2006. **151**(564-570).

41. Burdge, G.C. and S.A. Wootton, *Conversion of alpha-linolenic acid to EPA, DPA and DHA in your women.* British Journal of Nutrition, 2002. **88**: p. 411-420.

42. Burdge, G.C., A.E. Jones, and S.A. Wootton, *Eicosapentanaeoic and docosapentanaenoic acid are the principal products of alpha-linolenic acid metabolism in young men.* British Journal of Nutrition, 2002. **88**: p. 355-363.

43. Bemelmans, W.J., et al., *Increased alpha-linolenic acid intake lowers C-reactive protein, but has no effect on markers of atherosclerosis.* European Journal of Clinical Nutrition, 2004. **58**(7): p. 1083-9.

44. Kim, H.K., S. Choi, and H. Choi, *Suppression of hepatic fatty acid synthase by feeding alpha-linolenic acid rich perilla oil lowers plasma triacylglycerol level in rats.* Journal of Nutritional Biochemistry, 2004. **15**(8): p. 485-92.

45. Brody, T., *Nutritional Biochemistry*. 2 ed. 1999, New York: Academic Press. 1006.

46. Hopkins, D.T. and F.H. Steinke, *Effect of Water of Hydration on the Measurement of the Protein Efficiency Ratio of Casein and Soybean Protein with Rats.* J. Nutr., 1976. **106**(10): p. 1438-1446.

47. Prior, R.L. and G. Cao, *In vivo total antioxidant capacity: comparison of different analytical methods1.* Free Radical Biology and Medicine, 1999. **27**(11-12): p. 1173-1181.

48. Pellegrini, N., et al., *Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays.* Journal of Nutrition, 2003. **133**(9): p. 2812-9.

49. Bruckdorfer, K.R., *Antioxidants and CVD.* Proceedings of the Nutrition Society, 2008. **67**(2): p. 214-22.

50. Beaglehole, R., et al., *Decreased blood selenium and risk of myocardial infarction.* International Journal of Epidemiology, 1990. **19**(4): p. 918-22.

51. Riemersma, R.A., et al., *Plasma antioxidants and coronary heart disease: vitamins C and E, and selenium.* European Journal of Clinical Nutrition, 1990. **44**(2): p. 143-50.

52. Gresch, E.E.M.D., *Vitamin E Consumption and the Risk of Coronary Disease in Women.* Journal of Occupational Medicine November, 1993. **35**(11).

53. Gresch, E.E.M.D., *Vitamin E Consumption and the Risk of Coronary Heart Disease in Men.* Journal of Occupational Medicine November, 1993. **35**(11).

54. Stampfer, M.J., et al., *Vitamin E consumption and the risk of coronary disease in women.[see comment].* New England Journal of Medicine, 1993. **328**(20): p. 1444-9.

55. Rimm, E.B., et al., *Vitamin E consumption and the risk of coronary heart disease in men.[see comment].* New England Journal of Medicine, 1993. **328**(20): p. 1450-6.

56. Yochum, L.A., A.R. Folsom, and L.H. Kushi, *Intake of antioxidant vitamins and risk of death from stroke in postmenopausal women.[see comment].* American Journal of Clinical Nutrition, 2000. **72**(2): p. 476-83.

57. Stephens, N.G., et al., *Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS).* Lancet March, 1996. **347**(9004): p. 781-786.

58. Hennekens, C.H., et al., *Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease.[see comment].* New England Journal of Medicine, 1996. **334**(18): p. 1145-9.

59. Gissi-Prevenzione Investigators, *Dietary Supplementation With n-3 Polyunsaturated Fatty Acids and Vitamin E After Myocardial Infarction: Results of the Gissi-Prevenzione Trial.* Journal of Cardiopulmonary Rehabilitation March/April, 2000. **20**(2): p. 131.

60. Gissi-Prevenzione Investigators, *Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial.* Lancet August, 1999. **354**(9177): p. 447-455.

61. *Salba: a superior source of antioxidants*, Salba Group.

62. American Diabetes, A., *Postprandial blood glucose. American Diabetes Association.[see comment].* Diabetes Care, 2001. **24**(4): p. 775-8.

63. American Diabetes, A., *Diagnosis and classification of diabetes mellitus.* Diabetes Care, 2005. **28 Suppl 1**: p. S37-42.

64. Alberts, B., et al., *Molecular Biology of the Cell*. 4 ed. 2002, New York: Garland Science.

65. Ferraris, R.P., *Dietary and developmental regulation of intestinal sugar transport.* Biochemical Journal, 2001. **360**(Pt 2): p. 265-76.

66. Raben, A., et al., *Decreased postprandial thermogenesis and fat oxidation but increased fullness after a high-fiber meal compared with a low-fiber meal.* American Journal of Clinical Nutrition, 1994. **59**(6): p. 1386-94.

67. Murray, R.K., et al., *Harper's Illustrated Biochemistry*. 26 ed ed. 2003, New York, USA: McGraw-Hill companies.

68. DeFronzo, R.A., *Pathogenesis of type 2 diabetes mellitus.* Medical Clinics of North America. **88**(4): p. 787-835.

69. *Dietary Guidelines for Americans*. 2006, US Department of Health and Human Services.

70. Aroda, V.R. and R.R. Henry, *Incretin hormones in diabetes and metabolism.* 2004, Medscape.

71. Giugliano, D., A. Ceriello, and K. Esposito, *Glucose metabolism and hyperglycemia.* American Journal of Clinical Nutrition, 2008. **87**(1): p. 217S-222S.

72. Brownlee, M., *Biochemistry and molecular cell biology of diabetic complications.* Nature, 2001. **414**(6865): p. 813-20.

73. Unwin, N., et al. *Impaired glucose tolerance and impaired fasting glycemia: the current status on definition and intervention*. in *International Diabetes Federation IGT/ IFG Consensus Statement. Report of an Expert*

Consensus Workshop 1-4. 2001. Stoke Poges, UK.

74. Decode Study Group, t.E.D.E.G., *Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria.* Archives of Internal Medicine, 2001. **161**(3): p. 397-405.

75. Coutinho, M., et al., *The relationship between glucose and incident cardiovascular events. A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years.[see comment]*. Diabetes Care, 1999. **22**(2): p. 233-40.

76. Hanefeld, M., et al., *Risk factors for myocardial infarction and death in newly detected NIDDM: the Diabetes Intervention Study, 11-year follow-up.* Diabetologia, 1996. **39**(12): p. 1577-83.

77. Chiasson, J.L., et al., *Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial.[see comment]*. Lancet, 2002. **359**(9323): p. 2072-7.

78. Chiasson, J.L., et al., *Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial.[see comment]*. Jama, 2003. **290**(4): p. 486-94.

79. *Definition and diagnosis of diabetes mellitus*

and intermediate hyperglycemia: report of a WHO/IDF consultation. 2006, World Health Organization: Geneva.

80. Yorek, M.A., *The role of oxidative stress in diabetic vascular and neural disease.* Free Radical Research, 2003. **37**(5): p. 471-80.

81. Brownlee, M., *The pathobiology of diabetic complications: a unifying mechanism.* Diabetes, 2005. **54**(6): p. 1615-25.

82. Ceriello, A., et al., *Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: the possible role of hyperglycemia.* Metabolism: Clinical & Experimental, 1999. **48**(12): p. 1503-8.

83. Anderson, J.W., et al., *Effects of psyllium on glucose and serum lipid responses in men with type 2 diabetes and hypercholesterolemia.* American Journal of Clinical Nutrition, 1999. **70**(4): p. 466-73.

84. Jenkins, D.J., et al., *The glycaemic index of foods tested in diabetic patients: a new basis for carbohydrate exchange favouring the use of legumes.* Diabetologia, 1983. **24**(4): p. 257-64.

85. Brouns, F., et al., *Glycaemic index methodology.* Nutrition Research Reviews June, 2005. **18**(1): p. 145-171.

86. Wolever, T.M.S., *The Glycaemic Index: A Physiological Classification of Dietary Carbohydrate*. 2006: Cabi Publishing. 272.

87. Kamphuis, M.M., M.S. Westerterp-Plantenga, and W.H. Saris, *Fat-specific satiety in humans for fat high in linoleic acid vs fat high in oleic acid.* European Journal of Clinical Nutrition, 2001. **55**(6): p. 499-508.

88. Chau, C.F., Y.L. Huang, and M.H. Lee, *In vitro hypoglycemic effects of different insoluble fiber-rich fractions prepared from the peel of Citrus sinensis L. cv. Liucheng.* Journal of Agricultural & Food Chemistry, 2003. **51**(22): p. 6623-6.

89. Brand-Miller, J., et al., *Low-glycemic index diets in the management of diabetes: a meta-analysis of randomized controlled trials.[see comment].* Diabetes Care, 2003. **26**(8): p. 2261-7.

90. Stevens, J., et al., *Dietary fiber intake and glycemic index and incidence of diabetes in African-American and white adults: the ARIC study.* Diabetes Care, 2002. **25**(10): p. 1715-21.

91. Hodge, A.M., et al., *Glycemic index and dietary fiber and the risk of type 2 diabetes.[see comment].* Diabetes Care, 2004. **27**(11): p. 2701-6.

92. Schulze, M.B., et al., *Glycemic index, glycemic load, and dietary fiber intake and incidence of type 2 diabetes in younger and middle-aged women.[see comment].* American Journal of Clinical Nutrition, 2004. **80**(2): p. 348-56.

93. Kelly, S., et al., *Low glycaemic index diets for coronary heart disease.* Cochrane Database of Systematic Reviews, 2004(4): p. CD004467.

94. Opperman, A.M., et al., *Meta-analysis of the health effects of using the glycaemic index in meal-planning.* British Journal of Nutrition, 2004. **92**(3): p. 367-81.

95. Aller, R., et al., *Effect of soluble fiber intake in lipid and glucose levels in healthy subjects: a randomized clinical trial.* Diabetes Research & Clinical Practice, 2004. **65**(1): p. 7-11.

96. McIntosh, M. and C. Miller, *A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus.* Nutrition Reviews, 2001. **59**(2): p. 52-5.

97. Nishimune, T., et al., *Glycemic response and fiber content of some foods.* American Journal of Clinical Nutrition, 1991. **54**(2): p. 414-9.

98. Edwards, C.A., *The Physiological Effects of Dietary Fiber*, in *Dietary Fiber in Health and Disease*, D. Kritchevsky and C. Bonfield, Editors. 1995, Eagan Press: St. Paul. p. 58-71.

99. Jenkins, D.J., et al., *Unabsorbable carbohydrates and diabetes: Decreased post-prandial hyperglycaemia.* Lancet, 1976. **2**(7978): p. 172-4.

100. Pick, M.E., et al., *Oat bran concentrate bread products improve long-term control of diabetes: a pilot study.* Journal of the American Dietetic Association, 1996. **96**(12): p. 1254-61.

101. Anderson, J.W., *Cholesterol-Lowering Effects of Soluble Fiber in Humans*, in *Dietary Fiber in Health and Disease*, D. Kritchevsky and C. Bonfield, Editors. 1995, Eagan Press: St. Paul. p. 126-136.

102. Samra, R.A. and G.H. Anderson, *Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men.* American Journal of Clinical Nutrition, 2007. **86**(4): p. 972-9.

103. Schenk, S., et al., *Different glycemic indexes of breakfast cereals are not due to glucose entry into blood but to glucose removal by tissue.* American Journal of Clinical Nutrition, 2003. **78**(4): p. 742-8.

104. Weickert, M.O., et al., *Cereal fiber improves whole-body insulin sensitivity in overweight and obese women.* Diabetes Care, 2006. **29**(4): p. 775-80.

105. Weickert, M.O., et al., *Impact of cereal fibre on glucose-regulating factors.* Diabetologia, 2005. **48**(11): p. 2343-53.

106. Jenkins, D.J., et al., *Dietary fibres, fibre analogues, and glucose tolerance: importance of viscosity.* British Medical Journal, 1978. **1**(6124): p. 1392-4.

107. Jenkins, D.J. and T.M. Wolever, *Slow release carbohydrate and the treatment of diabetes.* Proceedings of the Nutrition Society, 1981. **40**(2): p. 227-35.

108. Benini, L., et al., *Gastric emptying of a solid meal is accelerated by the removal of dietary fibre naturally present in food.* Gut, 1995. **36**(6): p. 825-30.

109. Holt, S., et al., *Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol.* Lancet, 1979. **1**(8117): p. 636-9.

110. Ximenes, H.M., et al., *Propionate inhibits glucose-induced insulin secretion in isolated rat pancreatic islets.* Cell Biochemistry & Function, 2007. **25**(2): p. 173-8.

111. Boillot, J., et al., *Effects of dietary propionate on hepatic glucose production, whole-body glucose utilization, carbohydrate and lipid metabolism in normal rats.* British Journal of Nutrition, 1995. **73**: p. 241-251.

112. Asplund, J.M., et al., *The effect of intragastric infusion of glucose, lipids or acetate on fasting nitrogen excretion and blood metabolites in sheep.* British Journal of Nutrition, 1985. **54**(1): p. 189-95.

113. Jenkins, D.J., W.M. Hunter, and D.V. Goff, *Ketone bodies and evidence for increased insulin secretion.* Nature, 1970. **227**(5256): p. 384-5.

114. Vuksan, V., J. Blom, and A.S. Lee, *Measurement of viscosities of ground flax and Salba*. 2007, University of Toronto: Toronto.

115. Howarth, N.C., E. Saltzman, and S.B. Roberts, *Dietary fiber and weight regulation.* Nutrition Reviews, 2001. **59**(5): p. 129-39.

116. Welch, I.M., et al., *Duodenal and ileal lipid suppresses postprandial blood glucose and insulin responses in man: possible implications for the dietary management of diabetes mellitus.* Clinical Science, 1987. **72**(2): p. 209-16.

117. Gannon, M., et al., *Effect of added fat on plasma glucose and insulin response to ingested potato in individuals with NIDDM 10.2337/diacare.16.6.874.* Diabetes Care, 1993. **16**(6): p. 874-880.

118. Owen, B. and T.M. Wolever, *Effect of fat on glycaemic responses in normal subjects: a dose-response study.* Nutrition Research, 2003. **23**: p. 1341-1347.

119. Cunningham, K.M. and N.W. Read, *The effect of incorporating fat into different components of a meal on gastric emptying and postprandial blood glucose and insulin responses.* British Journal of Nutrition, 1989. **61**(2): p. 285-90.

120. Gatti, E., et al., *Differential effect of unsaturated oils and butter on blood glucose and insulin response to carbohydrate in normal volunteers.* European Journal of Clinical Nutrition, 1992. **46**(3): p. 161-6.

121. Richieri, G.V., et al., *Fatty acid binding proteins from different tissues show distinct patterns of fatty acid interactions.* Biochemistry, 2000. **39**(24): p. 7197-204.

122. Rasmussen, O., et al., *Differential effects of saturated and monounsaturated fat on blood glucose and insulin responses in subjects with non-insulin-dependent diabetes mellitus.* American Journal of Clinical Nutrition, 1996. **63**(2): p. 249-53.

123. MacIntosh, C.G.H.S.H.B.-M., J.C., *The degree of fat saturation does not alter glycemic, insulinemic or satiety responses to a starch staple in healthy men.* Journal of Nutrition, 2003. **133**(8): p. 2577-80.

124. Joannic, J.L., et al., *How the degree of unsaturation of dietary fatty acids influences the glucose and insulin responses to different carbohydrates in mixed meals.* American Journal of Clinical Nutrition, 1997. **65**(5): p. 1427-33.

125. Sigalet, D.L. and G. Martin, *Lymphatic absorption of glucose and fatty acids as determined by direct measurement.* Journal of pediatric surgery, 1999. **34**(1): p. 39-43.

126. Gentilcore, D., et al., *Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes.* Journal of Clinical Endocrinology & Metabolism, 2006. **91**(6): p. 2062-7.

127. Collier, G. and K. O'Dea, *The effect of coingestion of fat on the glucose, insulin, and gastric inhibitory polypeptide responses to carbohydrate and protein.* American Journal of Clinical Nutrition, 1983. **37**(6): p. 941-4.

128. Collier, G.R., et al., *The acute effect of fat on insulin secretion.* Journal of Clinical Endocrinology & Metabolism, 1988. **66**(2): p. 323-6.

129. Dworatzek, P.D., R.A. Hegele, and T.M. Wolever, *Postprandial lipemia in subjects with the threonine 54 variant of the fatty acid-binding protein 2 gene is dependent on the type of fat ingested.* American Journal of Clinical Nutrition, 2004. **79**(6): p. 1110-7.

130. Ercan, N., M.C. Gannon, and F.Q. Nuttall, *Effect of added fat on the plasma glucose and insulin response to ingested potato given in various combinations as two meals in normal individuals.* Diabetes Care, 1994. **17**(12): p. 1453-9.

131. Gannon, M.C., et al., *The insulin and glucose responses to meals of glucose plus various proteins in type II diabetic subjects.* Metabolism: Clinical & Experimental, 1988. **37**(11): p. 1081-8.

132. Karamanlis, A., et al., *Effects of protein on glycemic and incretin responses and gastric emptying after oral glucose in healthy subjects.* American Journal of Clinical Nutrition, 2007. **86**(5): p. 1364-8.

133. Floyd, J.C., Jr., et al., *Secretion of insulin induced by amino acids and glucose in diabetes mellitus.* Journal of Clinical Endocrinology & Metabolism, 1968. **28**(2): p. 266-76.

134. Simpson, R.W., et al., *Macronutrients have different metabolic effects in nondiabetics and diabetics.* American Journal of Clinical Nutrition, 1985. **42**(3): p. 449-53.

135. Peters, A. and M. Davidson, *Protein and fat effects on glucose responses and insulin requirements in subjects with insulin-dependent diabetes mellitus.* Am J Clin Nutr, 1993. **58**(4): p. 555-560.

136. Nuttall, F.Q. and M.C. Gannon, *Metabolic response to egg white and cottage cheese protein in normal subjects.* Metabolism: Clinical & Experimental, 1990. **39**(7): p. 749-55.

137. Nuttall, F.Q., M.A. Khan, and M.C. Gannon, *Peripheral glucose appearance rate following fructose ingestion in normal subjects.* Metabolism: Clinical & Experimental, 2000. **49**(12): p. 1565-71.

138. Rask-Madsen, C. and G.L. King, *Mechanisms of Disease: endothelial dysfunction in insulin resistance and diabetes.* Nature Clinical Practice Endocrinology & Metabolism, 2007. **3**(1): p. 46-56.

139. Josse, A., *Almonds, Glycemic Excursions, Oxidative Stress and Risk Factors for Coronary Heart Disease*, in *Nutritional Sciences*. 2006, University of Toronto: Toronto. p. 170.

140. Poppitt, S.D., D. McCormack, and R. Buffenstein, *Short-term effects of macronutrient preloads on appetite and energy intake in lean women.* Physiology & Behavior, 1998. **64**(3): p. 279-285.

141. Flint, A., et al., *Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies.* International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 2000. **24**(1): p. 38-48.

142. Wolever, T., et al., *Day-to-day variation in glycaemic response elicited by white bread is not related to variation in satiety in humans*. 2008: Toronto.

143. Akhavan, T. and G.H. Anderson, *Effects of glucose-to-fructose ratios in solutions on subjective satiety, food intake, and satiety hormones in young men.* American Journal of Clinical Nutrition, 2007. **86**(5): p. 1354-63.

144. Anderson, G.H., et al., *Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men.* American Journal of Clinical Nutrition, 2002. **76**(5): p. 1023-30.

145. Cardello, A.V., et al., *Development and testing of a labeled magnitude scale of perceived satiety.* Appetite, 2005. **44**(1): p. 1-13.

146. Berthoud, H.R., *Vagal and hormonal gut-brain communication: from satiation to satisfaction.* Neurogastroenterology & Motility, 2008. **20 Suppl 1**: p. 64-72.

147. Bloom, S., *Hormonal regulation of appetite.* Obesity Reviews, 2007. **8 Suppl 1**: p. 63-5.

148. Chaudhri, O., C. Small, and S. Bloom, *Gastrointestinal hormones regulating appetite.* Philosophical Transactions of the Royal Society of London - Series B: Biological Sciences, 2006. **361**(1471): p. 1187-209.

149. Dhillo, W.S., *Appetite regulation: an overview.* Thyroid, 2007. **17**(5): p. 433-45.

150. Porrini, M., et al., *Evaluation of satiety sensations and food intake after different preloads.* Appetite, 1995. **25**(1): p. 17-30.

151. Naslund, E. and P.M. Hellstrom, *Appetite signaling: from gut peptides and enteric nerves to brain.* Physiology & Behavior, 2007. **92**(1-2): p. 256-62.

152. Mayer, J., *The glucostatic theory of regulation of food intake and the problem of obesity.* Bulletin, 1952. **14**(2): p. 43-9.

153. Mayer, J., *Bulletin of the New England Medical Center, Volume XIV, April-June 1952: The glucostatic theory of regulation of food intake and the problem of obesity (a review).* Nutrition Reviews, 1991. **49**(2): p. 46-8.

154. VanderWeele, D.A., D.R. Skoog, and D. Novin, *Glycogen levels and peripheral mechanisms of glucose-induced spppression of feeding.* American Journal of Physiology, 1976. **231**(6): p. 1655-9.

155. Campfield, L.A. and F.J. Smith, *Functional coupling between transient declines in blood glucose and feeding behavior: temporal relationships.* Brain Research Bulletin, 1986. **17**(3): p. 427-33.

156. Louis-Sylvestre, J. and J. Le Magnen, *Fall in blood glucose level precedes meal onset in free-feeding rats.* Neuroscience & Biobehavioral Reviews, 1980. **4 Suppl 1**: p. 13-5.

157. Gielkens, H.A.J.V., Marco; Lam, Wai F.; Lamers,Cornelis B.H.W.; Masclee, Ad A.M., *Effects of hyperglycemia and hyperinsulinemia on satiety in humans.* Metabolism, 1998. **47**(3): p. 321-324.

158. Pittas, A.G., et al., *Interstitial glucose level is a significant predictor of energy intake in free-living women with healthy body weight.* Journal of Nutrition, 2005. **135**(5): p. 1070-4.

159. Freeland, K. and T. Wolever, *Effects of cereal fiber Compared to Glycemic Carbohydrate on Appetite, Short-term Food Intake, Orocecal Transit Time and Blood Glucose [Abstract].* Am J Clin Nutr, 2002. **75**(2): p. 339S-439.

160. Holt, S.H., et al., *A satiety index of common foods.* European Journal of Clinical Nutrition, 1995. **49**(9): p. 675-90.

161. Holt, S.H., J.C. Brand Miller, and P. Petocz, *Interrelationships among postprandial satiety, glucose and insulin responses and changes in subsequent food intake.* European Journal of Clinical Nutrition, 1996. **50**(12): p. 788-97.

162. Zemel, M.B., et al., *Regulation of adiposity by dietary calcium.* FASEB Journal, 2000. **14**(9): p. 1132-8.

163. Westerterp-Plantenga, M.S., et al., *Dietary protein, metabolism, and body-weight regulation: dose-response effects.* International Journal of Obesity December, 2006. **30**(3): p. S16-S23.

164. Grimes, D.S. and C. Gordon, *Satiety value of wholemeal and white bread.* Lancet, 1978. **2**(8080): p. 106.

165. Turnbull, W.H., J. Walton, and A.R. Leeds, *Acute effects of mycoprotein on subsequent energy intake and appetite variables.* American Journal of Clinical Nutrition, 1993. **58**(4): p. 507-12.

166. Rolls, B.J.B., Elizabeth A.; Thorwart,Michelle L., *Water incorporated into a food but not served with a food decreases energy intake in lean women.* American Journal of Clinical Nutrition, 1999. **70**(4): p. 448-455.

167. Rolls, B.J., E.A. Bell, and B.A. Waugh, *Increasing the volume of a food by incorporating air affects satiety in men.* American Journal of Clinical Nutrition, 2000. **72**(2): p. 361-8.

168. Rolls, B.J.B., E A.Castellanos,V H.Chow,M.Pelkman,C L.Thorwart,M L., *Energy density but not fat content of foods affected energy intake in lean and obese women.* American Journal of Clinical Nutrition, 2008.

169. Papazian, R., *Bulking up fiber's healthful reputation: more benefits of "roughage" are discovered.* 1998, U.S. Food and Drug Administration.

170. Kay, R.M. and S. Stitt, *Food form, postprandial glycemia, and satiety.* American Journal of Clinical Nutrition, 1978. **31**(5): p. 738-9.

171. Qualmann, C., et al., *Insulinotropic actions of intravenous glucagon-like peptide-1 (GLP-1) [7-36 amide] in the fasting state in healthy subjects.* Acta Diabetologica, 1995. **32**(1): p. 13-6.

172. Roberts, S.B., *High-glycemic index foods, hunger, and obesity: is there a connection?* Nutrition Reviews, 2000. **58**(6): p. 163-9.

173. McMillan-Price, J. and J. Brand-Miller, *Low-glycaemic index diets and body weight regulation.* International Journal of Obesity December, 2006. **30**(3): p. S40-S46.

174. Holt, S., et al., *Relationship of satiety to postprandial glycaemic, insulin and cholecystokinin responses.* Appetite, 1992. **18**(2): p. 129-41.

175. Pereira, M.A. and D.S. Ludwig, *Dietary fiber and body-weight regulation. Observations and mechanisms.* Pediatric Clinics of North America, 2001. **48**(4): p. 969-80.

176. Ludwig, D.S., *Dietary Glycemic Index and Obesity.* J. Nutr., 2000. **130**(2): p. 280-.

177. Blundell, J.E. and R.J. Stubbs, *High and low carbohydrate and fat intakes: limits imposed by appetite and palatability and their implications for energy balance.* European Journal of Clinical Nutrition, 1999. **53 Suppl 1**: p. S148-65.

178. Blundell, J.E., et al., *The fat paradox: fat-induced satiety signals versus high fat overconsumption.* International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 1995. **19**(11): p. 832-5.

179. Liddle, R.A., et al., *Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction.* Journal of Clinical Investigation, 1985. **75**(4): p. 1144-52.

180. Lawton, C.L., et al., *The degree of saturation of fatty acids influences post-ingestive satiety.* British Journal of Nutrition, 2000. **83**(5): p. 473-82.

181. Chavez, M., et al., *Effect of a high-fat diet on food intake and hypothalamic neuropeptide gene expression in streptozotocin diabetes.* Journal of Clinical Investigation, 1998. **102**(2): p. 340-6.

182. Jones, P., P. Pencharz, and M. Clandinin, *Whole body oxidation of dietary fatty acids: implications for energy utilization.* Am J Clin Nutr, 1985. **42**(5): p. 769-777.

183. Erlanson-Albertsson, C. and J. Mei, *The effect of low carbohydrate on energy metabolism.* International Journal of Obesity, 2005. **29 Suppl 2**: p. S26-30.

184. Paddon-Jones, D., et al., *Protein, weight management, and satiety.* American Journal of Clinical Nutrition, 2008. **87**(5): p. 1558S-1561S.

185. Westerterp-Plantenga, M.S., et al., *Satiety related to 24 h diet-induced thermogenesis during high protein/carbohydrate vs high fat diets measured in a respiration chamber.* European Journal of Clinical Nutrition, 1999. **53**(6): p. 495-502.

186. Johnston, C.S., S.L. Tjonn, and P.D. Swan, *High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults.* Journal of Nutrition, 2004. **134**(3): p. 586-91.

187. Lejeune, M.P., et al., *Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber.* American Journal of Clinical Nutrition, 2006. **83**(1): p. 89-94.

188. Smeets, A.J., et al., *Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and peptide tyrosine-tyrosine concentrations following a single high-protein lunch.* Journal of Nutrition, 2008. **138**(4): p. 698-702.

189. Dahl, W.J., et al., *Effects of flax fiber on laxation and glycemic response in healthy volunteers.* Journal of Medicinal Food, 2005. **8**(4): p. 508-11.

190. Zeratsky, K., *Ground flaxseed: Better than whole?* 2008, Mayo Clinic.

191. Wong, S., K. Traianedes, and K. O'Dea, *Factors affecting the rate of hydrolysis of starch in legumes.* American Journal of Clinical Nutrition, 1985. **42**(1): p. 38-43.

192. Jenkins, D.J., et al., *Wholemeal versus wholegrain breads: proportion of whole or cracked grain and the glycaemic response.* Bmj, 1988. **297**(6654): p. 958-60.

193. Behall, K.M., D.J. Scholfield, and J. Hallfrisch, *The effect of particle size of whole-grain flour on plasma glucose, insulin, glucagon and thyroid-stimulating hormone in humans.* Journal of the American College of Nutrition, 1999. **18**(6): p. 591-7.

194. Haber, G.B., et al., *Depletion and disruption of dietary fibre. Effects on satiety, plasma-glucose, and serum-insulin.* Lancet, 1977. **2**(8040): p. 679-82.

195. DiMeglio, D.P. and R.D. Mattes, *Liquid versus solid carbohydrate: effects on food intake and body weight.* International Journal of Obesity & Related Metabolic Disorders: Journal of the International Association for the Study of Obesity, 2000. **24**(6): p. 794-800.

196. Lan-Pidhainy, X., *The effects of freezing and thawing on fiber-rich carbohydrate foods*, A.S. Lee,[Personal Communication]. 2008: Toronto.

197. Campfield, L.A., et al., *Human eating: evidence for a physiological basis using a modified paradigm.* Neuroscience & Biobehavioral Reviews, 1996. **20**(1): p. 133-7.

198. Burton, P. and H.J. Lightowler, *Influence of bread volume on glycaemic response and satiety.* British Journal of Nutrition, 2006. **96**(5): p. 877-82.

Appendices

Consent to Participate in a Research Study

Before agreeing to participate in this research study, it is important that you read and understand this research consent form. This form provides all the information we think you will need to know in order to decide whether you wish to participate in the study. If you have any questions after you read through this form, please address your study doctor or study personnel. You should not sign this form until you are certain that you understand everything on this form. You may also wish to discuss your participation in this study with your family doctor, a family member or a close friend. It is important that you are completely truthful with study personnel with respect to your health history and any medications you may be taking in order to prevent unnecessary harm to you if you decide to participate in this study.

Title of Research Study: The Effects of *Salvia hispanica*-enriched Foods on Glycemic Responses and Subjective Satiety

Investigators: Vladimir Vuksan, PhD
Professor, Department of Nutritional Sciences and Medicine
Faculty of Medicine, University of Toronto;
Associate Director, Risk Factor Modification Centre, St. Michael's Hospital
Phone: (416) 864-5525
E-mail: v.vuksan@utoronto.ca

Amy S. Lee BSc, MSc Candidate (under the supervision of Dr. Vuksan)
Department of Nutritional Sciences
Faculty of Medicine, University of Toronto
Phone: (416) 864-6060 ext. 3364
E-mail: amysanda.lee@utoronto.ca

Study Sponsor: Salba Nutritional Solutions, Inc.

Purpose of the Research

The purpose of this study is to determine if breads containing the whole grain *Salvia hispanica* lower blood glucose responses and decrease hunger compared to breads without the grain but with the same amount of carbohydrate. The study will also determine which amount and which form (whole or ground) of *Salvia hispanica* causes the greatest feelings of fullness. Furthermore, we will investigate if there is a relationship between blood insulin levels and fullness or satiety. This study will comprise a portion of Amy Lee's research for her Master's thesis.

Salvia hispanica is a whole grain grown in South America that has been consumed as early as 3500 B.C. It has also been recently demonstrated to be completely safe for human consumption. Its composition is similar to that of the common flaxseed.

Description of the Research

Approximately 30 subjects will be enrolled in this study and this study will be conducted at the Risk Factor Modification Centre, St. Michael's Hospital (70 Richmond St. East, Main Floor, Toronto, Ontario, M5C 1N8).

You will be asked to visit the clinic on 11 separate occasions to eat 11 different meals. The visits will be approximately 2 hours long. The meals will consist of a serving of bread 9 containing either no *Salvia hispanica* or various amounts of it. Visits must be at least 48 hours apart. Therefore, your participation should last from between 5-10 weeks.

For each visit, you will come to the clinic in the morning after having fasted for 10-12 hours. You will first have a finger prick blood sample taken and will fill out a questionnaire, then will be served a meal. At 15, 30, 45, 60, 90 and 120 minutes after the meal you will have additional finger prick blood samples taken and will complete a questionnaire at each of these times.

Potential Harms (Injury, Discomforts or Inconvenience)

Participation in this study carries a very low risk of injury. You may experience a fleeting stinging sensation when you receive a finger prick for a blood sample, and may experience some minor swelling at the sample site for one or two hours after the sample is taken. The risk of infection from having a finger prick blood sample taken is extremely low. It is possible that you could have an allergic reaction to the test meals; this, however, has never been documented.

Potential Benefits

There may be no direct benefit to you for participating in this study. However, the knowledge gained from this study may benefit others in the future.

Confidentiality and Privacy

The study investigators, (hereby referred to as "study personnel") are committed to respecting your privacy. No other persons will have access to your personal health information or identifying information without your consent, unless required by law. Any medical records, documentation, laboratory samples, or information related to you will be coded by study numbers to ensure that persons outside of the study (i.e., sponsors) will not be able to identify you. No identifying information about you will be allowed off site. All information that identifies you will be kept confidential and stored and locked in a secure place that only the study personnel will have access to. In addition, electronic files will be stored on a secure hospital or institutional network and will be password protected. It is important to understand that despite these protections being in place, experience in similar studies indicates that there is the risk of unintentional release of information. The principal investigator will protect your records and keep all the information in your study file confidential to the greatest extent possible. The chance that this information will accidentally be given to someone else is small.

National and Provincial Data Protection regulations, including the Personal Information Protection and Electronic Documents Act (of Canada) or PIPEDA and the Personal Health Information Protection Act (PHIPA) of Ontario, protect your personal information. They also give you the right to control the use of your personal information, including personal health information, and require your written permission for your personal information (including personal health information) to be collected, used or disclosed for the purposes of this study, as described in this consent form. You have the right to review and copy your personal information. However, if you decide to be in this study or chose to withdraw from it, your right to look at or copy your personal information related to this study will be delayed until after the research is completed.

Publication of Results

We may present this study at scientific conferences and we intend to write an article about this study for a scientific journal. You can ask us to send you a copy of the article when it is published by contacting Dr. Vladimir Vuksan, the principal investigator.

Reimbursement

You will be compensated $30 per session, for a total of $330 for all 11 sessions.

Compensation for Injury

If you suffer a physical injury as a direct result of the administration of study foods or study procedures, you may obtain medical care in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this form waive your legal rights nor relieve the investigator, sponsors or involved institutions from their legal and professional responsibility.

Participation and Withdrawal

Participation in any research study is voluntary. If you choose not to participate, you and your family will continue to have access to customary care at St. Michael's Hospital. If you decide to participate in this study you can change your mind at any time without giving a reason, and you may withdraw from the study at any time without any effect on the care you and your family will receive at St. Michael's Hospital.

Research Ethics Board Contact

If you have any questions about your rights as a research subject, you may contact:

Dr. Julie Spence
Chair, St. Michael's Hospital Research Ethics Board
Telephone (416) 864-6060 ext. 2557

The Effects of Salvia hispanica-enriched Foods on Glycemic Responses Responses and Subjective Satiety

Consent

I acknowledge that the research study described above has been explained to me and that any questions that I have asked have been answered to my satisfaction. I have been informed of the alternatives to participation in this study, including the right not to participate and the right to withdraw without compromising the quality of medical care at St. Michael's Hospital for me and for other members of my family. As well, the potential risks, harms and discomforts have been explained to me and I also understand the benefits (if any) of participating in the research study.

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

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

Participants Name	Participants Signature	Date

I, the undersigned, have fully explained the study to the above participant.

Investigator or Designate Name	Investigator or Designate Signature	Date

If Signed by Designate:

Position of Designate	Investigator's Signature	Date

INFORMATION FORM

Appendix II

Salvia hispanica /ACUTE STUDY

Subject #: _____

Initials: _____

All information provided in this questionnaire will be kept confidential and released only for the purposes of the present study

Family name: _____

First name and initials: _____

Mailing address: _____

_____

_____

Tel.: _____

Fax: _____

E-mail: _____

Gender: Male Female

DOB (dd/mm/yyyy): / /

Age: _____

Family Physician: _____

Office use only:

Ht (cm):

Wt (kg):

	High blood sugar	High blood pressure
Has your doctor ever told you that you have high blood sugar, high blood pressure? If yes, then please give details: when, how high, medications (Rx), complications, etc. Yes No	When:_____ How high: Fasting glucose:_____mmolL Post-meal glucose:_____mmol/L HbA_{1c} (glycosolated haemoglobin)_____% Rx:_____ _____ Complications:_____ _____	When:_____ How high: sBP/dBP:_____/_____ mmHg Rx:_____ _____ Complications:_____ _____
Does anyone in your family have diabetes, high blood pressure, or heart disease? If yes, then please describe, indicating how long they have had it and their relationship to you. Yes No	Mother Father Siblings Aunt/Uncle Grandmother/grandfather	Mother Father Siblings Aunt/Uncle Grandmother/grandfather
Do you take medications, herbs or supplements? If yes, then please describe, indicating types, brand names, doses, and times. Yes No		

Have you been diagnosed with any of the following? (If yes, please indicate onset date, treatment and current status- recovered/ active condition)

CONDITION	NO	YES	Onset date	Present status	
				Recovered	Active (please indicate treatment)
Malabsorption syndrome					
Crohn's					
Ulcerative colitis					
Stomach (gastric) ulcer					
Duodenal ulcer					
Intestinal parasites					
Diarrhea (> 2 liquid stools/day)					
Constipation (≥ 3 days duration)					
Heart disease					
Stroke					
Heart attack					
Arrhythmia					
Uncontrolled hypertension Systolic BP ≥ 140 Diastolic BP ≥ 90					
Blood clotting disorders					
Anaemia					
Kidney disease					
Psychiatric conditions					

CONDITION	No	Yes	Onset date	Present status	
				Recovered	Active (please indicate treatment)
Infectious hepatitis (B, C, D)					
Recently diagnosed infectious hepatitis A, E					
HIV/ AIDS					
Tuberculosis					
Cancer					
Thyroid disease Do you experience any of the following**:** **Fatigue** **Unexplained weight gain** **Dry skin and hair** **Depressed mood** **Cold intolerance** **Constipation** **Increased cholesterol?** **Nervousness/irritability** **Palpitations** **Heat intolerance** **Increased sweating** **Unexplained weight loss** **Insomnia**					
Pancreatic disease					
Diabetes					
Asthma					
Any food allergies					
Allergies to ginseng or wheat bran powder					
Any food intolerance					

Any other health problems? No Yes (please describe) _____
_____

Lifestyle and diet

Are you following a special diet? No Yes (please describe) _____
_____

Do you smoke? Yes No

If yes, how many cigarettes per day? < 10 cigarettes/ day
> 10 cigarettes /day

If you are a past smoker, how many cigarettes did you smoke per day and when did you quit?
_____

Please list type, duration and frequency of any regular exercise (including walking):
_____

Please indicate the number of alcoholic beverages (spirit 1.5 oz, beer 1 bottle, wine 1 200 ml glass) consumed per day:

< 3/day >3/ day

Please indicate the number of coffee drinks per day (1 cup = 1.5 fl.oz.) indicating the type of coffee consumed (filtered, espresso, boiled, etc.)

0-5 cups/ day 5-8 cups/day ≥ 9 cups/ day

Type of coffee:_____

WOMEN ONLY:

Are you post-menopausal? Yes No

Did you recently experience any of the following symptoms?

SYMPTOM	No	Yes	Onset date	Frequency	Duration	Severity (mild/ moderate/ severe)
Bloating						
Belching						
Flatulence						
Diarrhoea						
Excessive urination						
Nausea						
Headache						
Dizziness						
Insomnia						
Anxiety						
Disorientation						
Poor wound healing						
Excessive bleeding after cuts						
Impaired vision						
Heart flutters						
Joint pain						
Numbness						

Have you participated in a clinical trial within the last 3 months? Yes No

CLINICAL ASSESSMENT

Salvia hispanica/ Acute Study

Subject #: _____

Initials: _____

Date:_____

Treatment Code:_____

Anthropometry

Ht (cm):_____

Wt (kg):_____

BF(%): _____

Waist:Hip (cm:cm): _____

BF printout

START TIME:_____

FINISH TIME:_____

Time taken to consume test meal:_____

Preclinical information

Did you consume at least **150g** (6oz.) of carbohydrate on **each** of the three days previous to this test? This amount is equivalent to **3** servings of any of the following alone or in combination: 2 slices of bread, 1 cup of cooked rice/pasta, 1 medium potato, 1 bowl of cereal with milk, 1 glass of juice/soft-drink, 3 oranges/apples, or 1 bowl of ice cream. Yes No Are you fasting this morning? If yes, then please describe the last meal you consumed before beginning your fast. Yes No	Time Food item Quantity _____ _____ _____ _____
Did you take **any** medications (prescription, OTC, etc.), remedies, or supplements last night or this morning? If yes, then please describe Yes No	Type _____ Dose:_____ Time:_____
How long ago did you last (1) empty your bladder and/or (2) have a bowel movement?	(1) Last urination:_____hrs ago (2) Last Bowel movement:_____hrs ago
Did you do anything last night that is not part of your regular routine? This may include social activities, exercise, or use of alcohol, medications, or supplements. If yes, then please describe. Yes No	_____
How many hours of sleep did you have last night? Does this represent a typical amount? Yes No	_____ hrs
Did you do anything before the test this morning that is not part of your regular routine? This may include exercise or use of alcohol, medications, or supplements. If yes, then please describe. Yes No	_____
What was your mode of transportation to the clinic this morning? Is this different from other clinic mornings? Yes No	_____
How would you rate your current level of health/well-being. Please comment on anything unusual. Excellent Good Fair Poor	_____

Participant ID:
Time: FASTING (0 min)
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

Participant ID:
Time: 15 min
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

Participant ID:
Time: 30 min
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

Participant ID:
Time: 45 min
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

Participant ID:
Time: 60 min
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

Participant ID:
Time: 90 min
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

Participant ID:
Time: 120 minutes (your clock may say 2:00)
Date: _____

PHYSICAL QUESTIONNAIRE

These questions relate to your physical assessment at this time. Please rate your feelings by **placing a vertical line** across the line at the point which best reflects your present feelings.

1. How strong is your desire to eat?

Very weak _____ Very strong

2. How hungry do you feel?

Not hungry at all _____ As hungry as I have ever felt

3. How full do you feel?

Not full at all _____ As full as I have ever felt

4. How much do you think you could eat now?

Nothing at all _____ A large amount

SYMPTOMS	PRESENCE	SEVERITY	Comment
Bloating	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Belching	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Diarrhoea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Flatulence	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive urination	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Nausea	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Headache	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Dizziness	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Disorientation	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Anxiety	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Poor wound healing	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Excessive bleeding after cuts	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	
Other (specify): _____	Yes No	Low 1---------2---------3---------4---------5---------6---------7 High	

UNIVERSITY of GUELPH LABORATORY SERVICES

Report

NUTRITIONAL LABELING ANALYSIS

Client: St. Michael's Hospital
Address: 61 Queen Street East
Toronto, ON M5C 2T2
Tel: (416) 867-7450
Fax: (416) 867-7442
E-mail: v.vuksan@utoronto.ca
Attn: **Dr. Vladimir Vuksan**

Sample Received: June 29, 2001
Reported: July 16, 2001

NUTRITION FACTS Serving Size (100 g)	
Amount Per Serving:	**% Daily Value**
Calories 500	
Fat 28g	43%
Saturated Fat 5g + Trans Fat 0g	25%
Cholesterol 1mg	0%
Sodium 200mg	13%
Potassium 694mg	6%
Carbohydrate 40g	13%
Fibre 36g Soluble fibre 2.3g Insoluble fibre 33.6g	144%
Sugars 0g	
Protein 21g	
Vitamin A 0%	
Vitamin C 6%	
Ca 70%	
Fe 50%	

484

carb 40g

36x4 = 144

500

356

Saturated fat 16.4%; Mono-unsaturated fat 7.3%; Poly-unsaturated fat 76.3%

Should you have any questions regarding these results, do not hesitate to call. As always, your business is greatly appreciated!

Results approved by: **Dr. Harold Faulkner**

Contact: **Dr. Harold Faulkner**
Supervisor
Food and Dairy Chemistry
Tel: 519-767-6236
Fax: 519-767-6240
E-mail: hfaulkne@lsd.uoguelph.ca

UNIVERSITY of GUELPH LABORATORY SERVICES

Report

Submission #: L02-025674

Sample ID:		02-0289606
Submitter Sample ID:		SALBA SEEDS
Sample Type:		Seed
Sampling Date & Time:		
alanine	mg/g	10.33
% alanine	%	5.3
arginine	mg/g	19.84
% arginine	%	10.2
% aspartic acid	%	8.8
aspartic acid	mg/g	17.19
% cysteic acid	%	3.6
cysteic acid	mg/g	7.10
cystine	mg/g	3.68
% cystine	%	1.9
% glutamic acid	%	18.1
glutamic acid	mg/g	35.25
glycine	mg/g	9.79
% glycine	%	5.0
histidine	mg/g	5.68
% histidine	%	2.9
isoleucine	mg/g	7.18
% isoleucine	%	3.7
leucine	mg/g	13.77
% leucine	%	7.1
lysine	mg/g	9.99
% lysine	%	5.1
% methionine	%	2.1
Methionine	mg/g	4.02
Methionine Sulfone	mg/g	13.00
phenylalanine	mg/g	10.08
% phenylalanine	%	5.2
proline	mg/g	7.85
% proline	%	4.0
serine	mg/g	11.64
% serine	%	6.0
% sum amino acid profile	%	100
sum of amino acid weights	mg/g	195.23

Legend: NA - Not Analyzed, ND - Not Detected, UNS - Unsuitable, < MDL - Less than Method Detection Limit, TR - Trace

SM

Printed: September 03, 2002

Table 3: Nutrient Composition of Salba (Study sample) Seeds
Analyzed at the University of Toronto, Dr.David Jenkins Lab (Jun 12/01)

Nutrients in 100g of Seeds	Freeze dried basis (%)
Fat	33.10%
Total Carbohydrate	35.25%
Total Fiber	34.45%
Available Carbohydrate	0.8%
Protein	22.06%
Ash	4.32%
Moisture	5.32%

Table 4: Fiber Content of Salba (Study sample) Seeds
Analyzed at the Maxxam Analytics Inc. June 11/01

Nutrients in 100g	Prosky enzymatic/gravimetric
Total Fiber	32.2g
Soluble	2.3g
Insoluble	29.9g

May 20/02

Table 1 Fatty Acid Profile of White Chia Seeds

SALBA

Fatty Acid	~~Chia~~ Seeds
16:0	5.5 ±0.3
18:0	1.4 ±0.2
20:0	0.0 ±0.0
Sum SFA	6.9 ±0.5
18:1n-9	4.6 ±0.4
18:1n-7	0.5 ±0.0
20:1n-9	0.0 ±0.1
Sum MFA	5.2 ±0.5
18:3n-3	69.3 ±1.3
Sum n-3 PUFA	69.3 ±1.3
18:2n-6	18.6 ±0.4
Sum n-6 PUFA	18.6 ±0.4
n-3/n-6	3.7 ±0.2

69.3
+ 18.6
87.9

Data is expressed as mean % composition, ±SD, n=4 samples.
SFA, saturated fatty acid; MUFA, monunsaturated fatty acids;
PUFA, polyunsaturated fatty acids

Dr. S. CUNNANE LAB
NUTRIT. SCI.
U of T

Table 1: Fatty Acid Profile of Salba (Study sample) Seeds
Analyzed at the University of Toronto, Dr.Steven Cunnane Lab
(August 13/01)

Fatty Acid	
SUM SFA	7.8±1.5
18:1n-9	5.2±1.0
SUM MUFA	6.0±1.3
18:2n-6	19.1±0.8
SUM n-6 PUFA	19.1±0.8
18:3n-3	67.1±3.6
SUM n-3 PUFA	67.1±3.6
n-3/n-6	3.5±0.3

Data is expressed as mean % composition, ±SD, n=6 samples. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid