The Effect of Salvia hispanica L. (Salba) on Weight Loss in Overweight and Obese Individuals with Type 2 Diabetes Mellitus

by

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

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Master of Science
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Abstract

Canadian statistics indicate that the incidence of obesity is rising, and that the prevalence of type 2 diabetes mellitus (T2DM) within this group is significantly higher than those of a healthy weight. Preliminary evidence has shown that the oil-rich grain, Salvia hispanica L. (Salba), improves glycemic control, suppresses appetite, and affects additional cardiovascular disease (CVD) risk factors. This study followed a randomized, double-blind, placebo-controlled, parallel design in a sub-set population of twenty individuals who were overweight or obese and had T2DM. Participants received supplements of Salba, or an energy- and fibre-matched control, and followed a hypocaloric diet for 24 weeks. Findings of this study reveal that Salba does not significantly affect weight loss, glycemic control or other CVD risk factors. These findings are preliminary and highlight the complexities of weight loss research. Further investigation into the potential health benefits of Salba is currently being carried out.
I would like to thank my supervisor, Dr. Vladmir Vuksan, as well as Alexandra Jenkins for giving me the opportunity to complete my Masters in such an important field of research. The expertise and guidance they provided me with throughout my graduate studies is invaluable and I will undoubtedly continue to use the knowledge and skills they have instilled in me in my future endeavors. I would also like to thank my advisory committee members, Dr. Thomas Wolever and Dr. Pauline Darling, for providing me with both valuable feedback and encouragement.

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Without the dedication of the participants, this study would not have been possible. Their commitment, optimism and kindness has been truly inspirational to me and has been pivotal in my decision to continue to pursue a career in the field of medicine.

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<th>Description</th>
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<tbody>
<tr>
<td>%BF</td>
<td>Percent Body Fat</td>
</tr>
<tr>
<td>AI</td>
<td>Adequate Intake</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-Linolenic Acid</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine Aminotransferase</td>
</tr>
<tr>
<td>APPT</td>
<td>Activated Partial Thromboplastin Time</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate Aminotransferase</td>
</tr>
<tr>
<td>BIA</td>
<td>Bioelectrical Impedance Analysis</td>
</tr>
<tr>
<td>BF</td>
<td>Body Fat</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatinine</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>DER</td>
<td>Daily Energy Requirements</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual Energy X-Ray Absorptiometry</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaneoic Acid</td>
</tr>
</tbody>
</table>
EPA    Eicosapentanoic Acid
FBG    Fasting Blood Glucose
FFA    Free Fatty Acid
GLP-1  Glucagon-Like Peptide-1
GI     Glycemic Index
HbA1c  Glycated Hemoglobin
HC     Hip Circumference
HDL    High-Density Lipoprotein
hs-CRP High-Sensitivity C-Reactive Protein
HPLC   High Performance Liquid Chromatography
iAUC   Incremental Area Under the Curve
INR    International Normalized Ratio
IR     Insulin Resistance
LDL    Low-Density Lipoprotein
MI     Myocardial Infarction
n-3    Omega 3 Polyunsaturated Fatty Acid
n-6    Omega 6 Polyunsaturated Fatty Acid
NADH   Nicotinamide Adenine Dinucleotide
ORAC   Oxygen Radical Absorbance Capacity
PER    Protein Efficiency Ratio
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPG</td>
<td>Postprandial Glycemia</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin Time</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic Blood Pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SRD</td>
<td>Sucrose Rich Diet</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>TAC</td>
<td>Total Antioxidant Capacity</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>TG</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TSQ</td>
<td>Telephone Screening Questionnaire</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
</tr>
<tr>
<td>WC</td>
<td>Waist Circumference</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WHR</td>
<td>Waist-to-Hip ratio</td>
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Chapter 1
Introduction

1.1 Introduction

Obesity has become a problem of epidemic proportions in Western societies and around the globe. The increase in industrialization and urbanization throughout recent decades has been strongly associated with transformations in diet and lifestyle, specifically the consumption of more high-fat, energy dense foods and the predominance of sedentary behaviours (1). However, despite the abundance of lifestyle modification recommendations from health care professionals, the prevalence of overweight and obesity in North America continues to rise (2). Furthermore, obesity has been shown to be strongly linked to a variety of co-morbidities, specifically to the risk of developing type 2 diabetes mellitus (T2DM), although the mechanism of their relationship has yet to be fully elucidated.

Obesity is a multi-faceted problem, generated by both genetic and environmental factors. Consequently, its treatment is highly complex, and both surgical and pharmacological interventions have proven unsuccessful (3,4). Dietary modification remains a cornerstone in obesity management, and a variety of functional foods have been examined for their therapeutic use. Salvia hispanica L., commonly known as Salba, is an oil-rich grain that has demonstrated potential to act as one such food.

The current study investigated whether the use of Salba was effective and safe for the long-term management of obesity. The study followed a randomized, double-blind, placebo-controlled, parallel design in individuals who were overweight or obese and had T2DM. Effect on weight management was assessed by measuring weight as well as several known obesity-related risk factors, including percent body fat (%BF) and waist circumference (WC). Effects on glycemic
control were assessed by examining biochemical markers of glycated hemoglobin (HbA1c), fasting glucose and insulin. Additional metabolic parameters, that may be associated with changes in body weight, including lipids and inflammation, as well as blood pressure (BP) were also evaluated as markers of CVD risk.
Chapter 2
Literature Review

2.1 Obesity

Obesity is a growing epidemic throughout the world. The World Health Organization (WHO) defines overweight as having a Body Mass Index (BMI) between 25.0 and 29.9 kg/m². Clinical obesity is defined as having a BMI greater than 30 kg/m² and is further subdivided into 3 classes: Class I: 30.0-34.9 kg/m²; Class II: 35.0-39.9 kg/m²; Class III: ≥40.0 kg/m² (5). In 2008 it was estimated that 1.46 billion adults worldwide had a BMI of greater than 25 and that of these, over 500 million were clinically obese (6).

The obesity epidemic is particularly relevant in North America, where the prevalence of obesity is amongst the highest in the world. Data from the 2004 Canadian Community Health Survey revealed that 59.3% of the Canadian population is overweight and that 24.1% is clinically obese (2). These numbers depict a substantial increase from data collected in 1978/79, which showed that, at the time, only 13.8% of the Canadian population was obese (2).

Fundamentally speaking, obesity is a result of energy imbalance, where energy intake is greater than energy expenditure. However the causes of obesity are multi-factorial and highly complex. Environmental, social, and genetic factors have all been shown to play a vital role in the obesity epidemic, making its management and prevention extremely challenging (7).

The recent increase in industrialization and urbanization throughout North America has been associated with specific diet and lifestyle changes that can lead to obesity (1). Diets rich in high-fat energy dense foods and sugar-sweetened beverages, such as those found at popular fast-food restaurants, have been linked to both obesity and insulin resistance (IR) (8). Data from the U.S.
Department of Agriculture's Continuing Survey of Food Intakes by Individuals 1994-1996 revealed that 7% of Americans eat at a fast food restaurant on a daily basis (8). Data from the same survey also revealed that sedentary occupations and behaviours, specifically, television watching, is associated with a high BMI in both men and women (9). Being physically active has been shown to be highly protective against weight gain and its associated co-morbidities. Current Canadian guidelines suggest that adults aged 18-64 partake in 150 minutes of moderate-to-vigorous intensity aerobic physical activity per week (10). However, data collected from 2007-2009 in the Canadian Health Measures Survey indicated that only 15% of the population meets this recommendation (10).

In addition to its effects on personal health, obesity is having a detrimental effect on the Canadian health care system. Accounting for 2.4% of total health care expenditures in 1997, the continually growing obesity epidemic is placing a substantial burden on society as a whole (11).

2.1.1 Complications of Obesity

Obesity has been shown to be strongly associated with increased mortality and several co-morbidities including cardiovascular disease (CVD) and increased risk of type 2 diabetes mellitus (T2DM). Obesity has also been associated with many other health-related issues, such as psychosocial disturbances, osteoarthritis, obstetric complications, asthma and an overall impaired quality of life (12).

2.1.1.1 Cardiovascular Disease

Many components of the metabolic and inflammatory states generally associated with obesity act as predictors for a variety of cardiovascular complications. Dyslipidemia, a component of obesity characterized by low levels of high-density lipoprotein (HDL), high levels of low-density
lipoprotein (LDL) and raised plasma triglycerides (TG), has been demonstrated to predispose one to atherosclerosis, a thickening of the arterial walls that can lead to a myocardial infarction (MI) or a stroke (13). Data from the Framingham Heart Study, a population-based epidemiologic study of over 5,000 men and women, showed that there was a doubling in the risk of heart failure for subjects who were obese, as compared to normal weight subjects (14).

Data from multiple population studies have shown the strong and clear relation between BMI and hypertension, a component of CVD. According to data from the Framingham Study, obesity by itself accounts for approximately 78% and 65% of essential hypertension in men and women, respectively (15). The Nurses’ Health Study, which involved 80,000 women, revealed that weight gain of 5kg was associated with a 60% higher relative risk of developing hypertension, compared to those women who gained ≤2kg (16). Fortunately, even modest weight reduction may lead to meaningful reduction in blood pressure. In a meta-analysis of 25 RCTs the authors concluded that a 1kg loss of body weight was associated with an approximate 1 mmHg drop in blood pressure. Furthermore, in the Trial of Hypertension Prevention, a 2kg weight loss over a 6-month period resulted in a decline of 3.7 mmHg in systolic blood pressure (SBP) and a decline of 2.7 mmHg in diastolic blood pressure (DBP) (17).

Obesity has also been shown to increase the risk of major cardiac events by increasing markers of oxidative stress and systemic inflammation, such as high-sensitivity C-reactive protein (hs-CRP) (12).

2.1.1.2 Type 2 Diabetes Mellitus

Obesity has also been strongly linked to the risk of developing type 2 diabetes mellitus. It has been projected that for each kilogram of weight gained annually over 10 years, the risk of
developing T2DM over those 10 years rises by 49% (18). Associations of obesity with decreased glucose tolerance, alterations in glucose and insulin homeostasis, reduced metabolic clearance of insulin, and decreased insulin-stimulated glucose disposal have all been reported (19). In a prospective cohort study of 114,281 women, the risk of developing T2DM increased exponentially with increasing BMI. After 14 years of follow-up, women in the highest BMI group were significantly more likely to develop diabetes than those in the lowest BMI group (20). Findings of this study also showed that even modest weight gain in women after the age of 18 is associated with a twofold increase in diabetes risk (20).

Data from the 2004 Canadian Community Health Survey verified that a high BMI is a strong risk factor for T2DM. Only 2% of men whose BMI was within normal range (18.5 kg/m² ≤ BMI ≤ 24.9 kg/m²) reported having diabetes; this amount doubled in overweight men (24.9 kg/m² ≤ BMI ≤ 29.9 kg/m²) and nearly tripled among those who were clinically obese (BMI ≥ 30.0 kg/m²). The relationship between BMI and self-reported T2DM was similar in women (2).

2.1.1.2.1 Pathophysiology of T2DM

In individuals with T2DM insulin target tissues, namely adipose, liver and muscle, exhibit a decreased ability to respond to insulin, a hormone that is responsible for glucose uptake and storage. This stimulates the β-cells of the pancreatic islets of Langerhans to increase insulin production, resulting in hyperinsulinemia (21). Consequently, β-cell function becomes progressively exhausted, causing a subsequent crash in insulin levels and resulting in an insufficient amount of insulin available for normal blood glucose removal. Furthermore, due in large part to hepatic insulin insensitivity, the production of hepatic glucose continues, leading ultimately to hyperglycemia (22,23). Hyperglycemia, maintained over extended periods of time, has been reported to lead to microvascular complications, including retinopathy, nephropathy,
and neuropathy, as well as macrovascular complications, such as CVD and stroke (22). In the Heart Outcomes Prevention Evaluation study, CVD-related events were shown to be responsible for more than 40% of deaths among individuals with diabetes (24).

2.1.1.2.2 Diagnosis and Management of T2DM

According to the World Health Organization, T2DM is diagnosed in individuals with a fasting blood glucose (FBG) $\geq 7.0$ mmol/l (126 mg/dl) or a 2-hr plasma glucose of $\geq 11.1$ mmol/l (200 mg/dl) (25). In 2000 it was estimated that 171 million individuals worldwide met this criteria (25). The Clinical Practice Guidelines, released by the Canadian Diabetes Association (CDA) in 2008, set out a recommend target for glycated hemoglobin (HbA1c), a marker of long-term glycaemic control, of $\leq 7.0\%$ (26). However, data from the National Health and Nutrition Examination Survey revealed that, despite an average national improvement in glycaemic control, over 40% of Canadians diagnosed with T2DM remain above this target (27).

The primary goal of diabetes management is to attain glycemic control. This can be achieved through a variety of treatment options, including lifestyle modifications, such as a healthy diet and a regular exercise regimen (26), and/or by using pharmacological therapy. Medication, most often in the form of oral anti-hyperglycemic agents, can be used to increase the amount of insulin secreted by the pancreas, increase the sensitivity of target tissues to insulin, decrease the rate at which glucose is taken up from the GI tract, or suppress glucose production in the liver (28). When used in combination, the insulin secretogogue, sulphonylurea, and the insulin sensitizer, metformin, have been demonstrated to result in the greatest reductions of HbA1c, lowering it by 1.5-2.0% (29).
2.1.1.2.3 Obesity and T2DM

Although the connection between obesity and T2DM has been well established, the cause and effect relationship has yet to be fully elucidated. It remains unknown whether it is obesity that causes an increase in insulin resistance (IR), or whether it is elevated insulin levels that results in obesity. Generally, the greater the severity of obesity, the higher the fasting and postprandial serum insulin concentrations (30,31).

Free Fatty Acids (FFAs) underlie one of the primary mechanisms through which obesity is believed to lead to IR. Lipotoxicity, a phenomenon observed in obese individuals, where high levels of FFAs are released from adipocytes into circulation, may promote the development of T2DM (8). Accumulation of FFAs in adipocytes, β-cells, muscle, liver, and arterial tissues impairs insulin signalling and several of the intracellular steps of glucose metabolism. This leads to compensatory hyperinsulinemia and is proposed to play a role in the pathogenesis of β-cell dysfunction and muscle and liver IR (32).

Obesity is also characterized by inflammation (33). In fact, total body fat (BF) is the primary determinant of circulating inflammatory marker levels, such as hs-CRP (34). Levels of hs-CRP have been shown to be high in both individuals who are obese and in individuals who have T2DM (34). Furthermore, in a more recent study, it was found that hs-CRP levels were higher in obese individuals with diabetes compared to those with diabetes alone (35). This can be attributed to common pathways involved in the development of inflammation and metabolic diseases. The intracellular signalling pathways activated by inflammatory responses have been shown to interfere with insulin signalling. Thus, in obese individuals, where there is over-expression of inflammatory markers, insulin signalling is inhibited, leading to IR (36).
In addition, the adipose tissue of obese mice has been shown to secrete pro-inflammatory and pro-thrombotic cytokines, including resistin, Interleukin-6, TNF-α, plasminogen activator inhibitor-1, and angiotensinogen (32). These cytokines have been shown to promote IR, contributing to the development of insulin-related metabolic disorders, such as T2DM (32). Furthermore, obesity has been shown to be associated with reduced leptin sensitivity and a decrease in adiponectin production. The effect on both of these gut hormones independently results in a reduction in insulin sensitivity (32).

On the other hand, insulin resistance and insulin hypersecretion may play a role in the pathogenesis of obesity. In an insulin resistant state, glucose uptake is reduced in muscle and liver cells, while, concurrently, there is increased hepatic glucose output (37,38). During this time, the adipose tissue retains some insulin sensitivity and it is suggested that unused nutrients are shunted to there. In the adipose tissue the nutrients are stored in adipocytes, which consequently grow in size and number, leading to obesity (37). This is known as the thrifty gene hypothesis, which is based on the principle that the human metabolism is genetically programmed to store nutrients in times of abundance in order to survive throughout periods of food scarcity (8).

Despite the knowledge gap in the mechanisms linking obesity and diabetes, extensive literature consistently supports weight loss as a viable treatment option for T2DM. Following voluntary weight loss, individuals with T2DM often have diminished clinical symptoms and reduced medication, particularly if it is maintained for the long-term (39). Evidence suggests that a reduction of 10% of total body weight can lead to a 30-40% reduction in diabetes-related complications and mortality (40,41). Therefore, the management of obesity may have a twofold benefit for individuals with T2DM.
2.1.2 Obesity Management

Considerable effort has been put towards pharmacological, surgical, dietary and behavioural interventions to manage obesity. Nevertheless, its prevalence continues to rise. A modest amount of steady weight loss, about 5-10% of initial body weight, can substantially improve obesity and its associated risk factors (42,43). Current therapy for overweight and obese individuals aims to reduce total body fat and to attain and maintain a healthy body weight for the long-term (7). Unfortunately, adherence to lifestyle approaches to obesity management is poor and current pharmacological therapies are limited in their efficacy and are often hampered by adverse events (44). Surgical interventions, despite resulting in clinically significant weight loss, can be highly invasive procedures and are recommended only for those with clinically severe obesity (BMI≥40kg/m²) (45).

2.1.2.1 Pharmacological Approaches to Weight Loss

The majority of anti-obesity medications work by reducing an individual’s appetite or by inhibiting fat absorption. The two most common medications that have been previously recommended for long-term treatment of obesity in Canada are Sibutramine (Meridia) and Orlistat (Xenical). Sibutramine is a neurotransmitter reuptake inhibitor. By blocking the uptake of noradrenaline and serotonin, it has been suggested that Sibutramine has both anorectic and thermogenic effects, which work simultaneously to reduce appetite (44). Randomized Controlled Trials (RCTs) conducted in overweight or obese individuals have shown that 34% of individuals taking Sibutramine experienced weight loss of 5% of their total body weight, and that 15% of individuals experienced weight loss of 10% of their total body weight, as compared to control (46). Although intake of Sibutramine has been shown to lower cholesterol and triglyceride
concentrations, it may also increase blood pressure (BP) and pulse (44). Sibutramine has also been shown to be associated with nausea, dry mouth, unusual tastes, insomnia, upset stomach, constipation, dizziness, and headache (46). In 2009 data gathered from the Sibutramine Cardiovascular OUTcomes Trial (SCOUT) revealed that the drug was associated with a higher rate of cardiovascular events (44). Consequently, the drug was withdrawn from the Canadian market.

Following the withdrawal of Sibutramine, Orlistat remains the only long-term anti-obesity drug that is available in Canada (45). Orlistat is a pancreatic lipase inhibitor that reduces fat absorption in the digestive tract (45). By preventing the hydrolysis of most triglycerides into FFAs, ingestion of Orlistat leads to increased fecal excretion of undigested fat (46,47). Several clinical trials have demonstrated both increased weight loss and reduced weight gain with Orlistat, as compared to placebo. A meta-analysis of 11 clinical trials showed that there was a 21% increase in the number of participants who achieved a 5% weight loss and an increase of 12% in the number of participants who achieved a 10% weight loss when taking Orlistat, compared to placebo (46,47). The mean weight loss after taking Orlistat for 12 months is 2.89kg (3). To date, there have been no demonstrated major safety concerns associated with taking Orlistat. However, the drug has been shown to cause unpleasant side effects, including loose stool, flatulence, fecal urgency, and fecal incontinence (47). Furthermore, as a result of its effects on fat absorption, Orlistat may reduce the absorption of ingested fat-soluble vitamins, such as Vitamins A, D, and E (45).

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that is secreted by entero-endocrine cells in the gastrointestinal tract. It functions to regulate the postprandial usage and storage of nutrients (48). When plasma glucose surpasses normal fasting levels, GLP-1 acts to regulate
insulin and glucagon secretion (49). Therefore, GLP-1 agonist therapy is primarily prescribed for the management of T2DM. However, GLP-1 has also been shown to delay gastric emptying, which promotes satiety and reduces subsequent food intake (48). Consequently, GLP-1 agonist therapy is becoming increasingly recognized as weight loss therapy. In a placebo-controlled trial in individuals with T2DM, participants taking Exenatide, a GLP-1 agonist, had significantly reduced HbA1c and a reduction in weight from baseline of 3.0 kg after 30 weeks (50). Individuals who remained on Exenatide for 82 weeks achieved a further reduction in weight from baseline of 5.3kg (50). Unfortunately, GLP-1 agonist therapy has not been shown to be beneficial for all patients, as some individuals, generally those with lower BMIs, do not respond to treatment (48).

Oral agents to treat obesity have been shown to have only modest efficacy, with weight loss of <5kg after 1 year of treatment (51). Due to their undesirable side effects and potential to cause adverse events, anti-obesity medications are only recommended by health care professionals when individuals are unable to achieve clinically significant weight loss with dietary and exercise therapy (7). Furthermore, intake of these drugs can cause dependence (52) and cessation of treatment usually results in individuals regaining lost weight (47).

2.1.2.2 Surgical Approaches to Weight Loss

Bariatric surgery has been demonstrated to be the most effective treatment for obesity (45). In 1991 the National Institute of Health Consensus Development Panel concluded that bariatric surgery is medically indicated in patients with a BMI > 40 kg/m² or, when high risk co-morbid conditions are present, a BMI > 35 kg/m² (53). Restrictive bariatric surgical procedures, such as gastric banding or sleeve gastrectomy, physically reduce the volume of the stomach lumen in order to limit caloric intake (45). On the other hand, malabsorptive bariatric procedures, such as
a biliopancreatic diversion, work by shortening the small bowel to reduce nutrient uptake (45).
Although restrictive procedures are usually simpler and less risky, malabsorptive procedures
have been shown to result in greater net weight loss (45). In the Swedish Obese Subjects trial
the effect of surgical versus conventional treatment of obesity was examined. 2010 obese
patients underwent gastric surgery, and 2037 were treated conventionally with lifestyle
interventions. Individuals in the surgical treatment group achieved significantly greater weight
loss at both 2 years and 10 years post intervention (54). Furthermore, the incidence of diabetes,
hypertriglyceridemia and hyperuricemia were also significantly reduced in the surgical
intervention group (54).
Nevertheless, bariatric surgery is a complex procedure and can present significant risk to the
patient. A meta-analysis conducted to assess the effectiveness and safety of surgical treatment of
obesity determined that adverse events occur in 20% of individuals who undergo bariatric
surgery (4). Furthermore, in a RCT it was shown that, although bariatric surgery may result in
greater weight loss over a one year period, similar improvements in long-term risk factors and
co-morbidities can be achieved using non-surgical lifestyle interventions (55).

2.1.2.3 Dietary Approaches to Weight Loss

Dietary therapy, as well as increased physical activity, with the goal to reach neutral or negative
energy balance, remains a fundamental intervention in the management of obesity (56). Current
guidelines recommend a diet that is planned to create a deficit of 500 to 1,000kcal/day as an
integral part of any therapy aimed at achieving a healthy weight loss of 0.5-1kg/week (7).
However, this is difficult to maintain in the long-term, as it relies strongly on self-discipline and
can lead to uncomfortable episodes of hunger and, consequently, weight regain (57,58).
Fad diets have emerged as a popular, but highly controversial, means to achieve weight loss. By focusing on the elimination of a specific food or food groups, fad diets are often advertised as a “quick fix” and consequently, weight loss associated with them is generally not maintained (59).

In a clinical study by Dasinger et al. (2005) the effectiveness of 4 popular weight loss diets, namely Atkins, Zone, Weight Watchers and Ornish, were compared (60). The nutrient goals for each diet are as follows: Atkins: 20g/day of carbohydrate with gradual increases to 50g/day; Zone: 40:30:30 ratio of calories from carbohydrates, fat and protein, respectively; Weight Watchers: 1200-1600kcal/day; Ornish: vegetarian diet with 10% of total calories from fat.

Results from this study indicated that after 12 months the average weight loss was 4.8kg for Atkins, 3.2kg for Zone, 4.9kg for Weight Watchers and 7.3kg for Ornish (60). However, despite their positive effect on weight loss, these diets have been repeatedly criticized for their low adherence rates and associated side effects (61). Specifically, the Atkins diet has been examined for its potential to increase risk factors associated with heart disease (62) and, in individuals with pre-existing kidney problems, may increase the potential for ketosis and protein toxicity (63).

Hunger and energy intake regulation is extraordinarily multidimensional and involves cognitive, environmental and physiological mechanisms that act both independently and synergistically. Sensory factors, which are generally associated with the physical characteristics of food, such as smell, taste, texture, and appearance, have been shown to have a considerable affect on an individual’s food choices (64). Furthermore, physiological processes that result as a response to the ingestion of food, such as gastric distention, glucose homeostasis, fatty acid metabolism and the action of appetite-related hormones, all play a role in the regulation of appetite.

An interventional approach that can influence multiple control mechanisms involved in appetite and food intake regulation, and that is easy to adhere to, may offer an effective means to assist
with weight loss. Functional foods are foods that contain health benefits beyond those attributed to their basic nutritional composition (65). With respect to obesity, this may include positive effects on energy expenditure and satiety (65). Given its unique composition, along with promising findings from preliminary trials, Salvia hispanica L. (Salba) may prove to be one such food.

2.2 Salvia hispanica L. (Salba)

2.2.1 Background and Classification

Salvia hispanica L., commercially known as Salba, is a white oil-rich grain that has been developed through selective breeding of the original black grain, commonly known as Chia. This grain has been used for thousands of years, most notably by the ancient Aztec civilization, who used it as both a food and remedy and referred to it as “Running Food” because of its ability to keep them energized throughout long trading expeditions (66). Chia, the mother crop of Salvia hispanica L., consists of over 80 different varieties with varying nutritional compositions. However, selective breeding of this crop has resulted in the cultivation of two registered white varieties, Sahi Alba 911 and 912, aptly referred to as Salba, which has been shown to have both a more consistent nutrient composition and a greater nutrient density than the original common Chia. Salba is currently grown exclusively in the mineral dense soils of coastal Peru and, once harvested, is stored in a climate-controlled environment in North America. At present, Salba is commercially available on the Canadian, USA, and New Zealand markets.
2.2.2 Nutritional Composition

Preliminary clinical data shows that Salba reduces postprandial glycemia, suppresses appetite, reduces waist circumference and affects additional CVD risk factors, suggesting its potential as a functional food in weight management. Salba’s potential to generate such health benefits may be a result of its unique composition, as some of its main components have been implicated in the regulation of body weight (Table 2-1). Salba is one of the richest natural whole-food sources of dietary fibre, a nutrient implicated in reduced feelings of hunger and lower risk of obesity. As well, the main type of fat found in Salba, namely omega-3 polyunsaturated fatty acid (n-3), has been reported to be among the most satiating of fats. Salba contains a significant proportion of protein, the most satiating of the macronutrients, and is high in calcium, a mineral that has been indicated for its weight loss properties. These naturally occurring nutrients, which are abundant in Salba, may act additively or synergistically to promote weight loss and consequently effect diabetes and cardiovascular health outcomes.
Table 2-1. The nutritional composition of Salvia hispanica L. (Salba)

<table>
<thead>
<tr>
<th>Nutritional Component</th>
<th>Salba (100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>528</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td>32.1</td>
</tr>
<tr>
<td>Omega-3s (g)</td>
<td>19.8</td>
</tr>
<tr>
<td>Total Carbohydrate (g)</td>
<td>36.4</td>
</tr>
<tr>
<td>Dietary Fibre (g)</td>
<td></td>
</tr>
<tr>
<td>Soluble (g)</td>
<td>35.2</td>
</tr>
<tr>
<td>Insoluble (g)</td>
<td>29.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>23.1</td>
</tr>
<tr>
<td>Calcium (mg)</td>
<td>650</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>8.5</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>330</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>810</td>
</tr>
</tbody>
</table>

*Nutritional laboratory analysis performed by Maxxam, 2011*
2.2.2.1 Carbohydrate

Salba is composed of 36.4% total carbohydrate, of which 97% is in the form of dietary fibre, making Salba one of the highest natural sources of dietary fibre. According to the American Association of Cereal Chemists, dietary fibres promote beneficial physiological effects, including laxation, blood cholesterol attenuation, and glucose control (67). Fibre has also been shown to reduce appetite and subsequent food intake through a variety of metabolic and mechanical mechanisms. Howarth (2001) reviewed 20 studies and concluded that an increase in dietary fibre intake of 14g/day produces a significant increase in post-meal satiety and a decrease in subsequent hunger, relative to control, resulting in a 10% reduction in overall energy intake and, consequently, a reduction in total body weight (68). Furthermore, in a Canadian study done in an Aboriginal population with very low fibre intake (1.2g/MJ), increases in fibre by 1 standard of deviation was associated with a 39% reduction in the risk of having T2DM (69).

The current national Adequate Intake (AI), which is the recommended average daily nutrient intake level, for fibre is 26g/d for females 9-18y, 25g/d for females 19-50y and 21g/day for females ≥51y. The AI for fibre is 31g/d, 38g/day and 30g/day for males aged 9-13y, 14-50y and ≥51y, respectively. (70). These recommendations stem from evidence demonstrating widespread benefits in risk reduction of chronic disease markers with fibre intake. However, in North America, individuals generally consume fewer than 15 grams of dietary fibre per day (70). It is speculated, however, that the reasons for low fibre intake in the general population may be partially due to the unpleasant side effects associated with its consumption. Since rapid increases in dietary fibre consumption can lead to unwanted effects, such as bloating, gas, and abdominal discomfort (71) long-term high fibre dietary regimens may be unattractive. Therefore, a dietary fibre model in which maximum health benefits can be obtained from a lower dose may be more
realistic. One potential method of obtaining maximum benefits with a smaller dose is by using fibres that have superior physicochemical properties, such as viscosity, water-holding capacity, and fermentability (72).

An additional property of fibre that is believed to regulate appetite and energy intake, and that is commonly used as a method of classification, is solubility. Soluble fibres, such as psyllium husk, guar, beta-glucan from oats and barley and naturally-occurring pectins in fruit, are hydroscopic and are able to form a gel in water (73). Insoluble fibres, such as cellulose and hemicellulose, do not form gels when mixed with water and pass through the digestive system relatively intact (74). Of the fibre present in Salba, 16% is soluble fibre and the remaining 84% is insoluble fibre. Insoluble fibre decreases food transit time in the small intestine, resulting in decreased food absorption and leading to an increase in the amount of undigested food particles that reach the distal intestine. This may cause an increase in the secretion of satiety hormones in the gut, such as GLP-1 (75). Soluble fibre, on the other hand, works by inducing mechanical effects on the gastrointestinal tract. Gastric distention, due to the swelling of soluble fibre together with food, creates a feeling of fullness by activating stretch receptors in the gut and initiating afferent vagal signals (68). Within 2-4 hours after a meal has been consumed the contents of the stomach are completely emptied into the duodenum in a process referred to as gastric emptying. Soluble fibre intake also delays gastric emptying, creating a feeling of fullness for a longer period of time (68). Lowered postprandial glycemia is another mechanism through which soluble fibre has been suggested to increase feelings of fullness. By delaying gastric emptying, soluble fibre intake slows the digestion and absorption of carbohydrates. This prevents sudden drops and wide swings in blood sugar levels, which, according to the Glucostatic Theory, are signals that would normally trigger hunger (76).
The Glucostatic Theory of food intake regulation suggests that a decline in plasma glucose concentration results in an increase in appetite (77,78). However, it is the rate of glucose utilization and the slope of the plasma glucose curve that appears to affect appetite and food intake, as oppose to the absolute levels of glucose at any particular time point (79). In healthy individuals, 30-60 minutes after the consumption of a carbohydrate-rich meal, plasma glucose typically rises to reach a peak and then falls back to baseline. This precise response has been found to vary depending on the source of carbohydrate and the rate at which it is digested (80).

The Glycemic Index (GI) is a tool used to classify the glycemic responses of various carbohydrates. It is measured as the incremental area under the curve (iAUC) of the blood glucose response elicited by a 50g available carbohydrate portion of a particular food expressed as a percentage of the iAUC of a reference food, usually anhydrous glucose (81). Rapidly absorbed carbohydrates are classified as high-GI foods and cause an immediate and large increase in blood glucose levels, requiring a great amount of insulin to be released. This leads to the rapid and quick removal of glucose and results in blood glucose levels that crash below the baseline level. Due to the counter-regulatory hormone response it has been shown to initiate, this fall below baseline is believed to stimulate hunger (82). Conversely, with the consumption of low-GI foods, the level of plasma glucose is stabilized and maintained above baseline for a longer duration, which is believed to prevent the feelings of hunger that accompany hypoglycemia (79). It has also been postulated that since low-GI foods are digested more slowly, more undigested starch reaches the ileum, promoting the release of satiety-signaling hormones (83).

Roberts (2003) examined the results of several experiments measuring satiety, hunger and/or food intake after low-GI and high-GI preloads. In each study there was lower subsequent energy intake following the low-GI meals. A meta-analysis of these studies revealed that there was an
81% greater energy intake after high-GI meals compared to low-GI meals (84). In addition, Ludwig (1999) concluded from 20 GI studies that low-GI meals consistently lower appetite and/or food intake (85).

In 1994 Slabber et al. randomized 30 obese women to follow either a high-GI or a low-GI hypocaloric diet. Not only did they conclude that more weight was lost on the low-GI diet, but fasting insulin and insulin:c-peptide ratio were also more significantly reduced in those on the low-GI diet, as compared to the high-GI diet (86).

Although Salba does not have a GI value per se, as it contains only 3% available carbohydrate, it has been shown that when added to white bread Salba has the ability to lower the glycemic response initiated by the bread (87). With this in mind, it can be hypothesized that the consumption of Salba may help increase satiety since its carbohydrate components promote the moderate and stable release of glucose into circulation and the induction of satiety signals.

2.2.2.2 Dietary Fat

Salba contains 32.1% fat, of which 62% is omega-3 polyunsaturated fatty acid (n-3), 17% is omega-6 polyunsaturated fatty acid (n-6), 6% is monounsaturated fatty acid and 11% is saturated fatty acid. Polyunsaturated omega-3 fatty acids include the 18-carbon alpha-linolenic acid (ALA), the 20-carbon eicosapentaneoic acid (EPA) and the 22-carbon docosahexaneoic acid (DHA). Animal products, such as fish, are excellent sources of EPA and DHA, whereas ALA is primarily found in plant foods, such as vegetables and seeds. The n-3 found in Salba is ALA.

Omega-3 polyunsaturated fatty acids are essential fatty acids, meaning that the body is unable to produce them. However, the body does possess limited ability to form EPA and DHA from ALA (88-90). This conversion process occurs competitively with omega-6 polyunsaturated fatty
acids, and therefore the ratio of n-6s to n-3s in the diet is of particular importance. It is estimated that the average North American consumes n-6s and n-3s in ratios between 14:1 and 20:1, which is significantly less than the recommended ratio of 4:1 (89). Therefore, given its approximate ratio of 1:3, Salba may help to counteract the imbalance of n-6s to n-3s in the typical Western diet.

Due to its high energy density and palatability (91,92), dietary fat is considered to be the least satiating nutrient in the short-term (93). Studies have shown that high-fat foods lead to greater passive food over-consumption, compared to low-fat, less energy-dense foods (94). However, polyunsaturated fat, which is the type of fat most abundant in Salba, has been reported to induce greater satiety than other types of fat. This can be attributed to the theory that the degree of fat saturation appears to affect satiety (95). In a study conducted by Lawton et al., the effects on appetite of three types of fat, namely monounsaturated, polyunsaturated, and saturated fat, incorporated into a meal were examined. Results from this study showed that the polyunsaturated fat meal increased post meal satiety to the greatest extent (95).

Intake of n-3s has also been associated with a lower risk of developing T2DM. In a prospective study examining 3088 men and women from the Cardiovascular Health Study, individuals with the highest concentrations of n-3s had a lower risk of developing diabetes (96). In the Singapore Chinese Health Study the association between total n-3s, marine n-3s (EPA, DHA), non-marine n-3s (ALA), total n-6s, and n-6: n-3 intake and risk of T2DM was examined in 43,176 individuals. Data from this study concluded that there was a significant inverse association between the consumption of non-marine sources of n-3s and incidence of T2DM (97).
2.2.2.3 Protein

Salba contains 23.1% vegetable protein with no amino-acid limiting factors for the adult diet. Thus, it contains all essential amino acids and is a complete and balanced source of protein (Table 2-2).

The quality of protein, sometimes expressed as a Protein Efficiency Ratio (PER), is dependent on the percentage of the protein that is likely to be used by the body (98). PER is measured by feeding rats a diet containing 9-10% of the given protein for a period of 4 weeks and subsequently calculating the weight gain per unit of protein consumed. Casein, derived from skim milk, has a PER of 2.5 and is used as the standard of comparison (99). The PER of Salba has been shown to be 91%, which is higher than that of soy protein, a highly regarded vegetable protein (100). Salba also does not contain any gluten, a protein often found in wheat products, and therefore it can be safely consumed by individuals with celiac disease (101).

Protein is the most satiating macronutrient, particularly for prolonged periods of time. Consumption of protein has been shown to reduce both appetite and food intake compared to the consumption of carbohydrate (102). Although carbohydrates, specifically simple carbohydrates, provide the most satiation immediately post consumption, protein provides greater feelings of fullness over longer time periods (103). In a long-term study by Johnston et al. (2004), participants were put on either a high-protein/low-fat diet or a high-carbohydrate/low-fat diet. After 4 weeks individuals who were in the high-protein group reported feeling more satiated and less hungry than those in the high-carbohydrate group (104). In another study, conducted by Lejeune et al. (2006), the effects of a high protein diet (30% of total energy intake) compared to an adequate protein diet (10% of total energy intake) was examined in a population of healthy females. The authors reported that the high protein diet, fed at energy balance for 4 days,
Table 2-2. The amino acid composition of Salba

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Salba (100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>314mg</td>
</tr>
<tr>
<td>Arginine</td>
<td>518mg</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>546mg</td>
</tr>
<tr>
<td>Cysteine</td>
<td>102mg</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>1080mg</td>
</tr>
<tr>
<td>Glycine</td>
<td>298mg</td>
</tr>
<tr>
<td>Histidine</td>
<td>174mg</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>218mg</td>
</tr>
<tr>
<td>Leucine</td>
<td>410mg</td>
</tr>
<tr>
<td>Lysine</td>
<td>288mg</td>
</tr>
<tr>
<td>Methionine</td>
<td>102mg</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>312mg</td>
</tr>
<tr>
<td>Proline</td>
<td>230mg</td>
</tr>
<tr>
<td>Serine</td>
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</tr>
<tr>
<td>Threonine</td>
<td>294mg</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>666mg</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>142mg</td>
</tr>
<tr>
<td>Valine</td>
<td>310mg</td>
</tr>
</tbody>
</table>

*Nutritional laboratory analysis performed at the University of Guelph*
significantly increased 24hr satiety, compared to the adequate protein diet (105). Although most noted for its long-term satiating effects, protein has also been shown to have strong satiating effects in the short term. Subjects consuming a meal with 25% protein reported significantly greater feelings of satiety at 30 and 120 minutes post meal consumption, compared to a meal containing 10% protein. In this study, satiety iAUC after 2 hrs was also shown to be significantly greater with the higher protein meal (106).

Several studies have suggested that diets which are high in protein may increase total weight loss and, more specifically, increase the percentage of fat loss (103). In a 6-month study fat loss was nearly doubled in subjects receiving a high-protein diet (25% of total energy intake) compared to subjects receiving a moderate-protein diet (12% of total energy intake) (107). High protein diets may also be beneficial for maintenance of weight loss. After a 6-month follow-up of 113 overweight men and women who lost 5-10% of their total body weight during a 4-week very-low-energy diet, those who consumed 18% of their energy intake as protein regained less weight than subjects who consumed only 5% of their energy intake as protein (108).

It has been suggested that diet-induced thermogenesis may be a factor that contributes to satiety (109). Increased oxygen consumption occurs during increased energy expenditure, and it is theorized that this oxygen deprivation creates feelings of satiety (110). Protein has been demonstrated to stimulate dietary-induced thermogenesis to a greater extent than other macronutrients (103). However, there is evidence to suggest that different protein sources elicit different levels of energy expenditure, and consequently may affect satiety to a different extent. It has been reported that animal protein causes a 2% higher energy expenditure than vegetable protein (110). Therefore, as the protein present in Salba is vegetable protein, it may not promote satiety as much as protein from an animal source would. Nevertheless, it can be hypothesized
that the addition of vegetable protein to a carbohydrate-rich meal would lower appetite to a greater extent than if an equicaloric amount of additional carbohydrate were to be added.

2.2.2.4 Minerals

In addition to being rich in important macronutrients, Salba also contains an abundance of other nutrients, including minerals such as calcium, iron, magnesium, potassium. In observational retrospective studies, dietary calcium has been shown to have a positive effect on energy metabolism and weight control (111). Data from the Health and Nutrition Examination Survey revealed a negative correlation between higher intakes of calcium and BMI (112). In a cross-sectional analysis of the Quebec Family Study, individuals were divided into three groups: those who consumed <600mg/d, those who consumed 600-1000mg/d, and those who consumed >1000mg/d of calcium. After controlling for daily energy intake, dietary protein, age and socioeconomic status, the results showed that women who consumed <600 mg/d had significantly greater body weight, BMI, %BF, and WC than those consuming >600mg/d (113). Several RCTs have recently been carried out in order to assess the effects of calcium intake on body weight and body composition in overweight individuals. In a placebo-controlled trial of 32 obese adults, subjects were randomized to a standard calcium diet (400-500mg/d), a high-calcium diet (800mg/d) or a high-dairy diet (1200-1300mg/d). In the high-calcium and high-dairy groups, weight loss was increased by 26% and 70%, respectively and fat loss was augmented by 38% and 64%, respectively (114).

The primary mechanisms underlying calcium’s influence on body weight are believed to be its ability to inhibit lipogenesis, increase lipolysis and attenuate the accumulation of lipids in adipocytes (115). Dietary calcium may also promote energy loss by reducing net energy absorption via the formation of calcium soaps in the gastrointestinal tract (111).
Many popular weight loss diets have been criticized for causing nutritional inadequacy and therefore cannot be recommended for populations (1). As Salba is abundant in both vitamins and minerals, particularly iron, magnesium, and potassium, its incorporation into a weight loss diet may prove to be particularly useful in ensuring that nutritional intake requirements are met.

### 2.2.2.5 Antioxidants

Antioxidants are substances that, when present at low concentrations compared to those of an oxidizable substrate, significantly prevent or delay oxidation of the substrate (116). A pro-oxidant, or reactive oxygen species (ROS), is a toxic substance that triggers substrate oxidation, causing damage to lipids, proteins and nucleic acids, resulting in various pathological events or diseases. The Total Antioxidant Capacity (TAC) of a compound is the ability of the compound to reduce pro-oxidants (116). TAC is equivalent to the sum of the Oxygen Radical Absorbance Capacity (ORAC) of both the lipophilic and hydrophilic components of the compounds, as determined with an ORAC assay (117). Salba has a TAC value of 84/g. This value is comparable to that of blueberries, which are considered to be an excellent source of antioxidants and have a TAC of 96/g. Other berries, such as the raspberry and strawberry, have TACs of 49/g and 36/g, respectively (118).

Numerous epidemiological studies have demonstrated an association between the consumption of antioxidant-rich foods, such as fruits and vegetables, with lowered risk of mortality from CVD events (119-121). Findings from the Cambridge Heart Association Study, a 2 year interventional trial, reported that consumption of Vitamin E, a fat-soluble antioxidant, reduced the risk of CVD-related events, including non-fatal MI (122). However, despite epidemiological findings, most RCTs show no protective effects of antioxidant supplementation on cardiovascular events (119,123,124).
2.2.3 Salba: Past Research

Several independent pre-clinical and clinical studies provide preliminary evidence that Salba may positively affect body weight, satiety, glucose metabolism and CVD risk factors, supporting rationale for further investigation into its ability to improve weight and cardiovascular health in individuals with T2DM.

2.2.3.1 Preclinical Studies

Based on data demonstrating the ability of black Chia seed, the original variety of Salvia hispanica L., to increase the n-3 content of animal products, including eggs, poultry meat and cow’s milk (125,126), Ayerza and Coates (2007) conducted a study on male Wistar rats to determine the effect of Chia seed on the animals’ plasma lipid composition (127). The dietary addition of Chia decreased the serum TG content and increased the HDL content of the rats. The rats that were fed Chia also had increased n-3 plasma contents and lower n-6 plasma contents, resulting in a lower n-6:n-3 ratio, which may reduce the risk of coronary heart disease (CHD) and other cardiovascular diseases (127).

In another preclinical study the effects of white Salba seeds on dyslipidemia, adiposity and insulin resistance were investigated in a rat model (128). All of the rats were fed a sucrose rich diet (SRD) for three months to induce metabolic syndrome. Subsequently, half of the animals continued with the SRD, while the other half started on a 2 month long diet where the original source of fat, maize oil, was replaced by Salba seeds (SRD + Salba). Results from this study showed that the addition of Salba into the diet significantly reduced visceral adiposity, lowered TG levels and reduced IR and dyslipidemia. Furthermore, weight gain in the rats fed the SRD + Salba diet was lower than in the rats who continued on the original SRD (128).
2.2.3.2 Clinical Studies

Based on the promising findings from the preclinical trials, several clinical studies examining the effects of Salba on various health parameters have been carried out. In a study by Vertommen et al. (2005) 12 healthy individuals consumed up to 50g of Salba per day for one month (129). Participants’ diastolic blood pressure significantly decreased from 66.1 ± 8.4 mmHg to 61.5 ± 7.0mmHg. Fasting serum triglycerides also decreased and a significantly decreased waist circumference was observed, but with no change in absolute weight. No side effects were reported and all safety parameters remained unchanged (129). Although the study lacked a control group, it presents promising clinical evidence that Salba may affect abdominal fat and warrants further research into the grain’s metabolic and cardioprotective effects.

In a single-blind, placebo-controlled RCT 20 individuals with well-controlled diabetes (HbA1c 6.0–8.5% and FBG 6.4–8.5 mmol/l) were given either ground Salba and Salba-enriched bread or wheat bran and an energy- and fibre-matched wheat bran-enriched bread to add into their regular diet for 12 weeks (130). Supplements were provided at a level of 15g/1,000 kcal intake and were calculated according to subjects’ individual daily energy requirements (DER). Results from this study demonstrated that dietary supplementation with approximately 37g/day of Salba significantly reduced SBP by 6 mmHg, clotting factors (von Willebrand Factor) by 21%, and low-grade body inflammation (hs-CRP) by 40% in a T2DM population, compared to a wheat bran control (130). Subjects continued their usual medications while on the study, and therefore the improvements seen were beyond conventional treatment. There were no beneficial long-term effects of the Salba treatment on fasting blood glucose or insulin and, although there was also no significant change in HbA1c compared to the control treatment, there was a significant change in HbA1c observed across the Salba treatment. These results may be attributed to the already
optimal baseline glycemic control (HbA1c 6.8 ± 0.9%) achieved by subjects’ underlying diabetes therapy. No adverse effects on renal, hepatic function and clotting time were observed with supplementation of Salba. However, observational findings indicated that a sizeable number of subjects on the Salba treatment group reported increased fullness after Salba consumption, suggestive of its potential to have satiating effects. However, the study protocol was designed to have no alterations in weight, precluding the assessment of any effect Salba might have on weight loss. The study, although preliminary, gave supporting evidence that Salba may have the direct potential to attenuate conventional and emerging CVD risk factors in T2DM and obesity.

In order to elucidate Salba’s potential mechanism of action an acute, double-blind, crossover, RCT was conducted to investigate the dose-response effect of Salba on appetite and glycemic control. Eleven healthy individuals received escalating doses: 0, 7, 14 and 24g of Salba baked into white bread. Blood glucose samples and subjective ratings of satiety, measures using a 100mm visual analog scale (VAS) were taken at 15-minute intervals post-consumption for a period of 2 hours. Compared to the white bread control, which was matched for available carbohydrates, a dose-response reduction of postprandial glycemia (PPG) was observed with all three doses of Salba. Specifically, there was a 41% reduction in iAUC for blood glucose with the highest-dose of Salba (24g). There was also a significant reduction in appetite ratings at 60, 90 and 120 mins post-consumption of the high dose, at 90 and 120 mins post-consumption of the intermediate dose, and at 120 mins post-consumption of the low dose, compared to control. Compared to the control bread, iAUC appetite ratings decreased after consumption of the low, intermediate and high doses by 41, 58 and 63%, respectively (87). Results from this study demonstrate that the addition of Salba to white bread has the potential to reduce appetite, possibly through its effects on PPG. In addition, these findings offer some explanation for the results observed in the previous long-term study on Salba whereby the reduction of
hyperglycemia may trigger a biochemical cascade resulting in the improvement of inflammation, hypercoaguability and reduction in BP in individuals with T2DM.

Most recently, a study was conducted by Neiman et al. (2010) to examine the effect of black Chia seed on weight loss and associated disease risk factors in overweight adults (131). Ninety participants ingested 25g of either Chia seed or placebo supplements mixed in 250mL of water before their first and last meals of every day for 12 weeks. Results of this study indicated that there was no significant change in body mass, body composition or various disease risk factor measures, including inflammation and blood pressure, in either of the treatment groups (131). Although this study suggests that Chia seeds do not play a role in weight management, there are several limitations to the design of this study that may have had significant effects on the study outcomes. Participants were instructed to maintain their regular dietary habits throughout the duration of the study. However, current guidelines state that in order to achieve measurable weight loss a diet plan with a deficit of 500-1000kcal/day is integral (7). Additionally, the Chia seed was administered immediately prior to regular meal consumption, without allowing significant time for its proposed physiological satiating effects to occur.

In summary, the aforementioned animal and human evidence provides rationale for undertaking a trial on the efficacy and safety of Salba on long-term weight loss in obese individuals with anticipation of preserving or improving participants’ health. The current study will investigate whether the addition of Salba to a hypocaloric diet will lead to stronger appetite suppression and greater weight reduction, compared to control, in addition to improvement of CVD risk factors in obese and overweight individuals with T2DM.
Chapter 3
Project Overview

3.1 Rationale

Canadian statistics indicate that the incidence of obesity is increasing at an alarming rate (2). Furthermore, the occurrence of type 2 diabetes mellitus, one of the most devastating health burdens faced worldwide, is 5-fold greater in obese individuals compared to those of a healthy weight, making weight control in this population particularly relevant (2). Preliminary preclinical and clinical data have shown that the oil-rich grain Salvia hispanica L., Salba, when consumed as a dietary supplement, may improve a variety of health outcomes.

Specifically, Salba has been shown to improve several obesity-related outcomes, suggesting its potential as a functional food in weight management. In rats, consumption of Salba significantly decreased visceral adiposity and serum triglycerides (128) and in humans, Salba significantly decreased waist circumference (129) and reportedly increased feelings of fullness (130). These findings suggest Salba’s potential for use as a novel therapy to treat, and potentially prevent, obesity. As modest weight loss has also been associated with improved glucose control in individuals with T2DM, the use of Salba may also have potential implications in diabetes control.

Although no significant effects were reported in a previous study conducted to examine the effect of black Chia seed on weight loss (131), this particular trial had some significant limitations in its study design. Therefore, in order to assess Salba’s ability to assist weight regulation, and potentially define a wider range of CVD and T2DM health benefits than has previously been indicated, further investigation is required.
3.2 Objective

The overall objective of this study is to explore the potential of Salba to affect weight loss and improve glycemic control and related cardiovascular disease risk factors, relative to control, when added to a hypocaloric diet in overweight and obese patients with type 2 diabetes mellitus.

The specific objectives are as follows:

Efficacy:

Primary: To assess the efficacy of Salba on weight loss, through assessment of body weight

Secondary: To assess the efficacy of Salba on glycemic control (HbA1c, fasting glucose and insulin)

Tertiary: To assess the efficacy of Salba on additional obesity-related outcomes (percent body fat [%BF], waist circumference [WC], waist-to-hip ratio [WHR]) and related CVD risk factors, including blood lipids (total cholesterol [TC], low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides [TG]), blood pressure (BP), and low grade body inflammation (high-sensitivity C-reactive protein [hs-CRP]),

Safety:

To monitor the effect of Salba on safety measures including kidney function (creatinine [Cr]), liver function (aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP]), bleeding time (prothrombin time [PT], activated partial thromboplastin time [APTT], international normalized ratio [INR]), and associated symptoms.
3.3 Hypothesis

Given the promising preclinical and clinical evidence demonstrated in previous studies (outlined in Chapter 2), it is hypothesized that the consumption of Salba will increase weight loss, to a greater extent than the control, will improve glycemic control and other CVD risk factors and will have no adverse effects on safety parameters.

Efficacy:

*Primary Hypothesis:* Consumption of Salba will have a greater effect on weight loss, relative to control.

*Secondary Hypothesis:* Salba consumption will have a favourable effect on glycemic control (improvement of HbA1c, fasting glucose and insulin)

*Tertiary Hypothesis:* Salba will improve additional obesity-related outcomes (%BF, WC, WHR) and CVD risk factors associated with weight management, including blood lipids, BP, and hs-CRP.

Safety: Consumption of Salba will not affect safety parameters, as indicated by kidney and liver function, and bleeding time, and will not be associated with adverse gastrointestinal side effects, as indicated through symptoms reporting.
Chapter 4  
Materials and Methods

4.1 Study Design

This study followed a randomized, placebo-controlled, double-blind, parallel design in individuals who were overweight or obese and had T2DM. The study took place at The Clinical Nutrition and Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada).

Clinical Trial Identifier: NCT01403571

4.2 Power Analysis

Given previous observations from weight loss studies in individuals with T2DM, to detect differences in weight loss of 6% (SD=11%) between two parallel groups with the power of 80% at a level of p<0.05, 54 participants per group (108 in total) would be required. Assuming a 22% attrition rate, a total of 132 subjects were to be enrolled.

4.3 Recruitment and Screening

Participants were recruited by contacting past volunteers at The Clinical Nutrition and Risk Factor Modification Centre, St. Michael’s Hospital, and through the use of advertisements published in local newspapers and posted throughout St. Michael’s Hospital. Individuals who were interested in participating in the research study were initially screened using a telephone screening questionnaire (TSQ) (Appendix 1). Eligible individuals were invited to The Clinical Nutrition and Risk Factor Modification Centre at St. Michael’s Hospital to attend an information session, where they were informed about the study details. Individuals were given as much time as they felt necessary to have all questions answered and were provided a copy of the consent
form (Appendix 2) to take home with them. They were instructed to contact the clinic staff if they were interested in participating in the study and to schedule a screening/run-in visit. After signing the informed consent form, individuals were further screened using anthropometric measurements as well as through completion of a detailed medical history (Appendix 3), a diet/lifestyle questionnaire (Appendix 4) and a physical activity questionnaire (Appendix 5).

4.4 Inclusion and Exclusion Criteria

**Inclusion Criteria:** Individuals with T2DM for at least 1 year treated with diet and/or oral hypoglycemic medications; HbA1c between 6.5% and 8.0%; between the ages of 35-75 years; having a BMI 25-40 kg/m².

**Exclusion Criteria:** Individuals who had weight change in the past three months >10% of total body weight; currently on insulin therapy; history of unstable angina, myocardial infarction or stroke (within 6 months), blood pressure >160mmHg/100mmHg, high fat diet (e.g. excess of 40% of energy from fat) and/or inappropriate eating pattern (nocturnal eating, binge eating, compulsive eaters, anorexia or bulimia); substantial psychological illness, including clinically-diagnosed depression; surgical procedures for weight loss and concomitant use of medication or supplements that alter body weight or appetite (including recent changes in weight-altering medications such as antidepressants, glucocorticoids, diuretics, laxatives, prescribed weight-loss medications such as Xenical or Meridia, or other investigational medications); substance abuse: alcohol (>2 drinks a day), nicotine substitutes or regular smoking, marijuana; taking supplements of ALA, dietary fibre, fish oil or consuming cold-water fish more than three times per week; the presence of any conditions which, in the opinion of the investigator, might jeopardize the health and safety of the subject or study personnel, or adversely affect the study results.
The study was approved by the St. Michael’s Hospital Research Ethics Board (Appendix 6). Randomization to treatment was done using a computer-generated random number table. Subjects were assigned to consecutive numbers after they provided written informed consent.

4.5 Study Intervention

The study treatment supplements consisted of either Salba (Salba Smart Natural Products LLC, Colorado, USA) or a control supplement. The control supplement consisted of 71.5% oat bran (PepsiCo, Peterborough, Canada), 19.7% inulin (Pure-le Natural, Barrie, Canada), and 8.8% maltodextrin (Whey-Factory.com, Canada) and was matched to the Salba in total energy and total dietary fibre (Table 4-1). The supplements were similar in appearance, taste, texture, and odor in order to minimize detectable differences between the treatments and maintain the double-blind study design.

Supplements were provided at a level of 30g of Salba/1000kcal intake, or 35.9g of energy- and fibre-matched control supplement (25.7g oat bran + 7.1g inulin + 3.2g maltodextrin) /1000kcal intake. This dose of Salba was selected as it is similar to the dose used in a previous long-term RCT conducted by Vertommen et al. (2005), where no side effects were reported (129). The Salba and control supplements were prepared in weekly pouches, labelled with unidentifiable codes, by an individual otherwise not involved in the study in order to ensure the blinding of study personnel and subjects. Subjects were instructed on how to incorporate the study supplements into their diet with the assistance of a recipe book/instruction manual (Appendix 7). Subjects were asked to return any non-consumed supplements at each follow-up visit in order for study compliance to be assessed. At each study visit subjects were provided with a new supply of supplements in a quantity sufficient to last them an additional seven days beyond the next scheduled visit.
Table 4-1. The nutritional composition of the study supplements

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salba</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving/1000kcal (g)</td>
<td>30</td>
<td>35.9</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>115</td>
<td>115</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>10.4</td>
<td>2.1</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>11.2</td>
<td>20.1</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>10.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>6.9</td>
<td>4.3</td>
</tr>
</tbody>
</table>
Subjects were instructed to follow a hypocaloric diet of -25% of their individual daily energy requirements (DER), which were calculated using the Harrison-Benedict equation multiplied by a “light” or “very light” activity factor of 1.2 or 1.375, as indicated in subjects’ physical activity questionnaires (Appendix 5). A minimum caloric intake was set at 1200 kcal/day. Subjects met regularly with a registered dietician who provided them with individualized dietary plans based on CDA’s Beyond the Basics: Meal Planning for Healthy Eating, Diabetes Prevention and Management, a meal planning guide that uses specific food groups and serving sizes to plan out daily meals (Appendix 8). Generally, subjects were encouraged to avoid excessive consumption of high-fat foods, reduce portion sizes, and increase their daily intake of fruits and vegetables.

4.6 Study Protocol and Timeline

Eligible subjects, as assessed by the participation criteria, were invited to attend the clinic to start the four week long run-in period, prior to which they were instructed on proper completion of 3-day food records (Appendix 9). During the run-in period, subjects were asked to maintain their usual lifestyle, including level of physical activity and diet in order to stabilize baseline measures. Individuals whose body weight decreased >2kg during the 4 week run-in phase were excluded from the study.

For the entire course of the study subjects were advised to stay on their current treatment medication regimen, as prescribed by their family doctor, and to report any changes in their medical status and treatment at each study visit.

During the 24-week treatment phase, subjects attended the clinic for follow-up visits at regular intervals for examination, as outlined in Figure 4-1. Subjects brought in a completed 3-day food record (Appendix 9) and completed a clinical assessment form (Appendix 10) at every visit.
The 3-day food records were examined in the presence of the subjects to minimize errors and clarify ambiguities and were used to assess dietary compliance, suggest personalized modifications, and re-emphasize specific dietary goals. Throughout the duration of the study the study investigators contacted the participants regularly in an effort to motivate participants and maximize diet compliance. In addition, participants were encouraged to contact the study investigators between visits to relieve any concerns that arose.

Due to the references in literature reporting on possible adverse effects of high fibre supplements, including bloating, constipation, flatulence and diarrhea (71), a questionnaire related to adverse effects was administered at every visit (Appendix 11).

**Figure 4-1. Study Timeline**

### 4.7 Study Measurements

#### 4.7.1 Anthropometric Assessment

At each visit anthropometric measurements were carried out, including height, weight, body composition, waist circumference (WC) and hip circumference (HC). Height was measured with a wall-mounted stadiometer (Perspective Enterprises, Portage, MI) with the subject’s head in the “Frankfurt horizontal” position and feet barefoot. The height measured was rounded to the
Table 4-2. Protocol of measurements conducted at each visit

<table>
<thead>
<tr>
<th>Measurement</th>
<th>-4</th>
<th>0</th>
<th>2</th>
<th>6</th>
<th>12</th>
<th>18</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Blood Sample</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Dual Energy X-Ray Absorptiometry</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Office Blood Pressure</td>
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<td>X</td>
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<td>X</td>
<td>X</td>
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<td>X</td>
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<td>X</td>
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<tr>
<td>3-Day Diet Record</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Symptoms Diary</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clinical Assessment Questionnaire</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
nearest centimeter. After emptying of the bladder and removing any excess clothing and shoes, the TANITA BC-418 Segmental Body Composition Analyzer (Arlington Heights, Illinois, USA) was used to measure weight, via a strain gauge load cell system, and percent body fat (%BF), via Bioelectrical Impedance Analysis (BIA). At the beginning and end of the study treatment phase body composition was also analyzed by a Dual Energy X-Ray Absorptiometry (DXA) scan using the Lunar Prodigy DF+10095. WC was measured using a non-stretchable measuring tape positioned at the most noticeable point of waist narrowing and was recorded to the nearest centimeter. Similarly, HC was measured with the tape measure positioned at the level of the widest point of the hip and recorded to the nearest centimeter.

4.7.2 Blood Samples

Blood samples were taken after a 10-12 hour overnight fast at the beginning, middle and end of the treatment phase. A phlebotomist withdrew blood from the forearm in SST, EDTA or citrate-treated vacuntainer tubes (BD Diagnostics, Quebec, Canada). All blood samples obtained from the forearm were analyzed by the Core Laboratory, St. Michael’s Hospital, Toronto, Canada using standard laboratory methodology.

4.7.2.1 Glycemic Parameters

Whole blood analysis of HbA1c was performed using high performance liquid chromatography (HPLC) with the Tosoh HLC-723 analyzer. A cation exchange column and gradient salt elution were used to separate HbA1c from HbA. HbA1c was expressed as a fraction of the total hemoglobin in the sample (132).

Serum glucose was analyzed using a reaction rate method with the Beckman Synchron LX System. Oxygen was consumed during the oxidation reaction of glucose at 37°C. The rate of
oxygen consumption occurred at the same rate as gluconic acid formation, and was directly proportional to the concentration of glucose in the sample (133).

Serum insulin was analyzed using immunoenzymatics with the Beckman Access Ultrasensitive Insulin Assay (Beckman Coulter, Brea, CA). Insulin was separated from the samples using immunoprecipitation with magnetic particles, and subsequently reacted with a chemiluminescent substrate to generate light (134). The light generated was directly proportional to the concentration of insulin in the sample, as measured using a luminometer and determined from a calibration curve (134).

### 4.7.2.2 Lipid Parameters

The Beckman SYNCHRON LX System was used to analyze serum total cholesterol (TC). This method determined cholesterol by a timed-endpoint method. Cholesterol esterase was used to hydrolyze cholesterol esters in the sample to free cholesterol and fatty acids (133). Cholesterol oxidase then oxidized free cholesterol, leading to the formation of hydrogen peroxide, which subsequently reacted to produce a coloured quinoneimine product (133). The change in absorbance, measured at 520nm, was directly proportional to the concentration of TC in the sample (133).

The Beckman SYNCHRON LX System was used to determine the concentration of serum triglycerides (TG) by a timed-endpoint method. Lipase was used to hydrolyze TGs in the sample to glycerol and FFAs (133). Three sequential enzymatic reactions with glycerolkinase, glycerophosphate oxidase and horseradish peroxidase then led to the formation of a red quinoneimine dye (133). The change in absorbance, measured at 520nm, was directly proportional to the concentration of TG in the sample (133).
High-density lipoprotein (HDL) was measured using the Beckman SYNCHRON LX System. HDL in the sample was first solubilized from HDL particles and then reacted with cholesterol esterase and cholesterol oxidase to generate hydrogen peroxide, which, in the presence of chromogens, produced a coloured product (133). The same detergent used for solubilization, also inhibited the reaction of cholesterol enzymes with low density, very-low density, and chylomicron lipoproteins (133). The reagent contained a polyanion that complexed low-density, very low-density and chylomicron lipoproteins and, in doing so, improved the selectivity for HDL (133). The change in absorbance, measure at 560nm, was directly proportional to the concentration of HDL in the sample (133).

Serum low-density lipoprotein was calculated using the Friedewald Formula:

\[
\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TG}/2.2)
\]  (135,136)

This equation is only valid when triglycerides are present at a concentration <4.52mmol/L (135). Therefore, LDL could not be calculated for TG \( \geq \) 4.52 mmol/L.

**4.7.2.3 Low-Grade Body Inflammation**

Serum high-sensitivity C-Reactive Protein (hs-CRP) was analyzed using the Beckman SYNCHRON LX System via turbidimetry. hs-CRP in combination with a specific antibody formed an insoluble antigen-antibody complex (133). The change in absorbance resulting from the formation of this complex, measured at 340nm, was proportional to the concentration of hs-CRP in the sample(133).
4.7.2.4 Safety Parameters

Serum aspartate aminotransferase (AST), alanine amino transferase (ALT), and alkaline phosphatase (ALP) activity were analyzed by a kinetic rate method using the Beckman SYNCHRON LX System. AST catalyzed the reversible transamination of L-aspartate and α-ketogluterate to oxaloacetate and L-glutamate. Subsequently, oxaloacetate was reduced to malate, while β-Nicotinamide Adenine Dinucleotide (NADH) was simultaneously oxidized to NAD⁺ (133). The rate of change in absorbance, measured at 340nm, was directly proportional to the activity of AST (133). In the presence of GPT the amino group of L-alanine was transferred to α-oxoglutarate to produce glutamate and pyruvate. The pyruvate was then converted by lactate dehydrogenase in the presence of NADH, which was oxidated to NAD⁺. The rate of oxidation of NADH, measured at 340nm, was directly proportional to the activity of ALT (133). In alkaline solution, the hydrolysis of p-nitrophenylphosphate produced p-nitrophenol, the rate of which, measured at 405nm, was directly proportional to the activity of ALP (133).

The SYNCHRON LX System was used to determine serum creatinine (Cr) concentration by the Jaffe rate method. Creatinine reacted with a reagent to produce a red colour complex. Absorbance readings were taken at 520nm between 19 and 25 seconds after the reaction began. The change in absorbance was used as a direct measure of the concentration of Cr in the sample (133).

Activated partial thromboplastin time (APTT) was analyzed using the Instrumental Laboratory ACL TOP by measuring the coagulation factors involved in the intrinsic pathway of coagulation, with the exception of platelet function (137). Factor XII was activated using a phospholipid reagent composed of lipids and an activator reagent (137).
The Instrumental Laboratory ACL TOP was used to analyze blood plasma for measurement of prothrombin time (PT). Tissue thromboplastin and calcium were added to activate the extrinsic pathway of coagulation (138). This resulted in the conversion of fibrinogen to fibrin and the subsequent formation of a solid gel (138). The time required for clot formation was measured as PT (138).

The International Normalized Ratio (INR) was calculated from PT and mean PT normal range of a control sample according to the following formula:

\[
\text{INR} = \frac{PT_{\text{test}}^{\text{ISI}}}{PT_{\text{normal}}} 
\]

(138)

Where ISI is the International Sensitivity Index based on the tissue factor used to activate the reaction (138).

4.7.3 Office Blood Pressure

Brachial blood pressure (BP) was assessed oscillometrically at every visit using the OMRON Digital Automatic Blood Pressure Monitor HEM-907 (Bannockburn, Illinois, USA). Prior to measurement, subjects remained seated in a quiet, temperature-controlled room for 5-10 minutes with their arm supported at heart level in order to achieve resting heart rate and BP. Subsequently, three readings were obtained from the brachial artery in the left forearm, with one minute separating each measurement. The arithmetic mean of the three readings was used in all analysis.
4.7.4 Compliance

4.7.4.1 Supplement Consumption

Supplement compliance was assessed by weighing out returned supplements and was calculated using the following formula:

\[
\text{Compliance} = \frac{\text{g of supplement consumed for } x \text{ days}}{\text{g of supplement prescribed for } x \text{ days}} \times 100
\]

Where \( x \) was the days of treatment (approximately 168)

4.7.4.2 Diet Analysis

Subjects completed a 3-day diet record at the beginning of the run-in period to receive training on how to properly complete the record. Subsequently, they completed a record prior to every study visit. Three-day food records (Appendix 9) obtained at the beginning, middle, and end of the treatment phase of the study were analyzed using ESHA Food Processor SQL, Version 9.8 (Salem, Oregon, USA). An average of the 3-day diet profile was generated for the analysis. Diets were analyzed for total caloric intake and macronutrient intake.

4.8 Statistical Analysis

Statistical analyses were performed using the Number Cruncher Statistical System (NCSS) 2000 software (NCSS Statistical Software, Kaysville, Utah). All data were adjusted for baseline values and tested for normality using the Shapiro-Wilk test. Subject characteristics were expressed as mean±standard deviation (SD), while all other data were presented as mean±standard error of the mean (SEM). Percent changes for each variable were based on the calculation for each individual
subject’s percent change. Comparison of differences from baseline to treatment-end in all parameters of efficacy, safety and compliance were assessed within treatment arms using a one-way ANOVA of repeated measures. Data was considered statistically significant at p<0.05.
Chapter 5
Results

5.1 Study Participants

Of the 164 individuals who contacted The Clinical Nutrition and Risk Factor Modification Centre at St. Michael’s Hospital wishing to participate in this study, 149 were telephone screened. From the 149 individuals that were telephone screened, 63 attended an information session, 51 of whom expressed further interest in participating in the study and provided informed consent. Out of the 51 subjects who were enrolled in the study and underwent a subsequent clinical screening visit, 10 did not meet further eligibility requirements. From the 41 subjects who met all of the eligibility requirements, 8 subjects were unable to make the time commitment, 4 subjects were lost to follow-up, 6 withdrew because of unrelated illness and 2 withdrew due to undesired side effects from the study material. Of the 21 subjects who completed the entire 24 week study protocol, 11 were randomized to the Salba treatment group and 10 were randomized to the control group. A detailed flow chart of subjects is presented in Figure 5-1.

As a result of low supplement compliance (<50%), the results from one subject in the Salba treatment group was excluded from analysis. The results presented herein are therefore for twenty subjects, 10 who were in the Salba treatment group and 10 who were in the control group. Baseline subject characteristics are presented in Table 5-1.

Analysis of baseline parameters revealed that the two groups were similar in all demographic and clinical parameters. Diabetes history and medication use for T2DM, cholesterol and hypertension were comparable between the two groups. Oral anti-hyperglycemic medications included


Figure 5-1. Subject flow from initial contact until study completion.
biguanides (Metformin), sulphonylureas (Glyburide, Glipizide) thiazolidinediones (Pioglitazone), dipeptidyl peptidase-4 inhibitors (Sitagliptin) and combinations of these (Janumet). Antihypertensive medications included angiotensin-converting enzyme inhibitors (Ramipril, Lisinopril, Perindopril), angiotensin II receptor antagonists (Diovan, Telmisartan), calcium channel blockers (Adalat, Amlopidine, Diltiazem), diuretics (Hydrochlorothiazide), direct renin inhibitors (Rasilez), β-blockers (Atenolol, Bisoprolol), α-adrenergic receptor agonists (Apo-Methyldopa) and combinations of these (Hyzaar, Avalide). Cholesterol medication used were statins (Crestor, Lipitor, Lescol). Three subjects in the Salba treatment group and 2 subjects in the control group were not taking any medication.

Anthropometric measures of weight, percent body fat (%BF) and waist circumference (WC) were also similar between the groups. However, baseline body mass index (BMI) was significantly higher (p=0.031) in the group randomized to the control treatment.

5.2 Treatment of Missing Data

Missing data values were as a result of failure of the Core Laboratory at St. Michael’s Hospital to analyze blood samples for all indicated tests. Missing values at baseline or end of treatment were recorded as intermediate values. Missing values during the intermediate visit were calculated using an average of baseline and end of treatment values. In addition, 2 subjects were missing LDL values. These could not be calculated with the algorithm used owing to TG levels that were ≥ 4.52 mmol/L.
Table 5-1. Baseline subject characteristics, presented as mean±SD

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>Treatment</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Salba</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sex (n)</td>
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<tr>
<td>Male</td>
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<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61.4±7.3</td>
<td>61.5±7.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.1±19.1</td>
<td>77.1±13.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.8±4.1</td>
<td>28.2±2.6</td>
</tr>
<tr>
<td>BF (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXA</td>
<td>42.7±8.0</td>
<td>41.9±7.2</td>
</tr>
<tr>
<td>BIA</td>
<td>38.5±6.2</td>
<td>35.1±6.9</td>
</tr>
<tr>
<td>WC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>114.3±10.1</td>
<td>100.3±9.0</td>
</tr>
<tr>
<td>Female</td>
<td>102.3±8.7</td>
<td>99.5±6.7</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>124.4±19.7</td>
<td>120.9±11.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>71.8±10.6</td>
<td>72.4±7.0</td>
</tr>
<tr>
<td>Medication Use (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CVD</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>BP</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3±0.9</td>
<td>6.9±0.6</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>7.7±1.5</td>
<td>7.1±1.2</td>
</tr>
</tbody>
</table>

BMI- Body Mass Index; BF- Body Fat; DXA- Dual Energy X-ray Absorptiometry; WC- Waist Circumference; BP- Blood Pressure; T2DM- Type 2 Diabetes Mellitus; CVD- Cardiovascular Disease; HbA1c- Glycated Hemoglobin; FBG- Fasting Blood Glucose
5.3 Efficacy of Salba

5.3.1 Effect on Primary Outcome Measure

5.3.1.1 Body Weight

Data for weight were normally distributed. For individuals in the Salba treatment group (n=10) weight decreased from (mean±SEM) 77.1±4.2kg at baseline to 76.3±4.2kg at week 12. However, compared to baseline, at week 24 weight increased by 0.35±0.85kg to 77.5±4.3kg. For individuals in the control group (n=10) weight decreased from 87.1±6.0kg at baseline to 86.8±6.3kg at week 12. However, at week 24 weight increased from baseline by 0.05±0.5kg to 87.1±6.3kg. By the middle of the study treatment period, week 12, the total body weight of individuals in the Salba treatment group decreased by 1.1±0.9% and the total body weight of individuals in the control group decreased by 0.48±0.48%. At end of study treatment period the total body weight of individuals in the Salba treatment group increased by 0.47±1.16% and the total body weight of individuals in the control group decreased by 0.1±0.6% (Figure 5-2). Statistical analysis failed to identify a significant difference in weight change, measured in kg, or percentage change of total body weight in either treatment group at any of the time points examined.
Figure 5-2. The effect of Salba on weight as a percentage of total body weight, compared to baseline, n=20. Results are presented as means with the SEM indicated by the vertical lines.
5.3.2 Effect on Secondary Outcome Measures

5.3.2.1 Glycated Hemoglobin

Data for glycated hemoglobin (HbA1c) were normally distributed. For individuals in the Salba arm (n=10) HbA1c decreased by 0.28±0.1% from 6.9±0.2% at baseline to 6.6±0.1% at week 12. However, by week 24 HbA1c returned to 6.9±0.3%. For individuals in the control group HbA1c increased 0.14%±0.3 from 7.3%±0.3 at baseline to 7.4±0.4% at week 12 and remained at 7.4±0.4% at week 24 (Figure 5-3). Analysis failed to demonstrate a significant difference between the two treatment arms and within the treatment groups at all time points.

5.3.2.2 Fasting Blood Glucose and Insulin

Analysis of the fasting serum glucose and fasting serum insulin data revealed that there were no significant differences between the Salba and control groups at any of the time points. There was also no significant difference within treatment groups at any time (Figure 5-4 and Figure 5-5)
Figure 5-3. The effect of Salba on HbA1c at middle and end of treatment, compared to baseline, n=20. Results are presented as mean±SEM.
Figure 5-4. The effect of Salba, compared to control, on fasting serum glucose levels at baseline, middle and end of treatment, n=20. Results are presented as mean±SEM.

Figure 5-5. The effect of Salba, compared to control, on fasting serum insulin levels at baseline, middle and end of treatment, n=20. Results are presented as mean±SEM.
5.3.3 Effect on Tertiary Outcome Measures

5.3.3.1 Obesity-Related Outcome Measures

5.3.3.1.1 Percent Body Fat

Data for %BF were normally distributed. When measured using BIA, the %BF of individuals in the Salba arm (n=10) decreased from 35.1±2.2 at baseline to 34.9±2.1 at week 12. However, by week 24 %BF increased to 35.8±2.4. For individuals in the control group %BF increased from 38.5±2.0% at baseline to 39.4±1.9% at week 12 and further increased to 39.5±2.1 by week 24 (Table 5-2). Analysis of change from baseline revealed no statistically significant differences between the Salba and control groups at any of the time points examined (Figure 5-6).

When measured using DXA, statistical analysis also failed to demonstrate a significant difference in change from baseline between the two treatment arms and within the treatment groups (Table 5-2).

**Table 5-2.** Comparing the effect of Salba to control on %BF at beginning, middle, and end of treatment. Results are presented as mean±SEM. For all parameters n=20.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
</tr>
<tr>
<td>%BF (BIA)</td>
<td>35.1±2.2</td>
<td>34.9±2.1</td>
<td>35.8±2.4</td>
</tr>
<tr>
<td>%BF (DXA)</td>
<td>41.9±2.3</td>
<td>N/A</td>
<td>40.5±1.6</td>
</tr>
</tbody>
</table>

BF- Body Fat; BIA- Bioelectrical Impedance Analysis; DXA- Dual Energy X-Ray Absorptiometry
Figure 5-6. The effect of Salba on %BF, measured by BIA, compared to baseline, n=20. Results are presented as mean±SEM.
5.3.3.1.2 Body Mass Index

There were no significant changes observed from baseline to treatment end in BMI in either of the treatment groups (Figure 5-7).

5.3.3.1.3 Waist and Hip Circumference

Analysis of waist circumference, hip circumference, and waist to hip ratio revealed that there were no significant differences between the Salba and control groups at any of the time points. There was also no significant difference within treatment groups at any time (Table 5-3).

Table 5-3. The effect of Salba, compared to control on waist circumference, hip circumference, and waist to hip ratio. Results are presented as mean±SEM. For all parameters n=20.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>P-value</th>
<th>Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
<td></td>
</tr>
<tr>
<td>WC (cm)</td>
<td>99.8±2.3</td>
<td>98.1±2.1</td>
<td>97.4±2.4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>105.9±3.3</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>105.6±1.3</td>
<td>105.1±1.3</td>
<td>106.2±1.3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>109.4±2.3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.95±0.02</td>
<td>0.93±0.01</td>
<td>0.92±0.02</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.97±0.02</td>
</tr>
</tbody>
</table>

WC- Waist Circumference; HC- Hip Circumference; WHR- Waist-to-Hip Ratio
Figure 5-7. The effect of Salba on body mass index, compared to baseline, n=20. Results are presented as mean±SEM.
5.3.3.2 Cardiovascular Disease Risk Factor Measures

5.3.3.2.1 Blood Pressure

Analysis revealed that there were no significant changes in either systolic blood pressure or diastolic blood pressure in either of the treatment groups (Table 5-4).

Table 5-4. The effect of Salba, compared to control, on BP at beginning, middle and end of treatment. Results are presented as mean±SEM. For all parameters n=20.

| Outcome Measure | Salba (n=10) | Control (n=10) | 
| Week 0 | Week 12 | Week 24 | Week 0 | Week 12 | Week 24 | 
| SBP (mmHg) | 120.9±3.5 | 121.3±4.1 | 125.6±4.2 | NS | 124.4±6.2 | 124.8±5.0 | 132±5.8 | NS |
| DBP (mmHg) | 72.4±2.2 | 71.3±2.5 | 74.2±2.3 | NS | 71.8±3.4 | 69.7±1.4 | 72.7±2.5 | NS |

SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure
5.3.3.2.2 Lipid Parameters

Analysis revealed that there were no significant changes in serum TC, HDL, LDL or TG in either treatment group. (Table 5-5)

Table 5-5. The effect of Salba, compared to control, on lipidemic parameters from baseline to treatment end. Results are presented as mean±SEM. For all parameters n=20, except LDL cholesterol for which n=18.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>4.6±0.4</td>
<td>4.9 ± 0.5</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.1±0.6</td>
<td>1.1±0.06</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2.7±0.4</td>
<td>2.8±0.4</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.9±0.4</td>
<td>1.6±0.3</td>
</tr>
</tbody>
</table>

TC- Total Cholesterol; HDL- High-Density Lipoprotein; LDL- Low-Density Lipoprotein; TG- Triglycerides

5.3.3.2.3 Low-Grade Body Inflammation

For individuals in the Salba treatment group (n=10), hs-CRP was 3.3±0.8 mg/L at baseline, 3.1±0.78 mg/L after 12 weeks, and 2.98±0.9 mg/L after 24 weeks. For individuals in the control group (n=10), hs-CRP was 4.78±1.8 mg/L at baseline, 6.22±2.2 mg/L after 12 weeks, and 5.49±2.6 mg/L after 24 weeks (Figure 5-8). Statistical analysis revealed that there were no significant differences between the two treatments groups or within the treatment groups at any of the time points examined.
**Figure 5-8.** The effect of Salba on low grade body inflammation at middle and end of treatment, compared to baseline, n=20. Results are presented as median±SEM.
5.3.4 Effect on Safety Parameters

5.3.4.1 Liver and Kidney Function

Statistical analysis comparing baseline to treatment-end revealed that there were no changes in the biochemical measures of kidney and liver function, shown in Table 5-6.

Table 5-6. Comparing the effect of Salba to control on hepatic and renal function from baseline to treatment end. Results are presented as mean±SEM. For all parameters n=20.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>P-value</th>
<th>Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>Week 0: 27.9±3.8</td>
<td>Week 12: 22.5±2.3</td>
<td>Week 24: 22.9±2.3</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ALP (U/L)</td>
<td>64.9±5.1</td>
<td>63.3±3.7</td>
<td>67.6±4.7</td>
</tr>
<tr>
<td></td>
<td>ALT (U/L)</td>
<td>28±4.5</td>
<td>24.7±3.0</td>
<td>27.4±3.5</td>
</tr>
<tr>
<td></td>
<td>Cr (μmol/L)</td>
<td>71.8±4.2</td>
<td>71.4±3.2</td>
<td>67.4±5.3</td>
</tr>
</tbody>
</table>

AST- Aspartate Aminotransferase; ALP- Alkaline Phosphatase; ALT- Alanine Amino Transferase; Cr- Creatinine

5.3.4.2 Bleeding Time

Statistical analysis comparing baseline to treatment-end revealed that there were no changes in the biochemical measures of bleeding time, shown in Table 5-7.
Table 5-7. Comparing the effect of Salba to control on bleeding time from baseline to treatment end. Results are presented as mean±SEM. For all parameters n=20.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>29.7±0.6</td>
<td>28.8±0.4</td>
<td>29.4±0.5</td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.3±0.2</td>
<td>11.4±0.2</td>
<td>11.3±0.2</td>
</tr>
<tr>
<td>INR</td>
<td>1.0±0.02</td>
<td>1.0±0.02</td>
<td>1.0±0.02</td>
</tr>
</tbody>
</table>

APTT- activated partial thromboplastin time; PT- prothrombin time; INR- international normalized ratio

5.3.4.3 Reported Symptoms

Presented in Table 5-8 are the side effects that were documented by subjects during the treatment phase of the study.

Table 5-8. Symptoms reported by subjects in symptoms diary

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Salba (n=10)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
</tr>
<tr>
<td>Bloating (n)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Belching (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flatulence (n)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Constipation (n)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Excessive Urination (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nausea (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Headache (n)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dizziness (n)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Anxiety (n)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Abdominal Pain (n)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>General Weakness (n)</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>
5.3.5 Compliance

5.3.5.1 Supplement Consumption

Individuals in the Salba treatment group were prescribed (mean±SD) 41.7±6.1g of Salba to consume daily. Individuals in the control group were prescribed 50.1±9.2g of the energy- and fibre-matched control supplement to consume daily. For the first 12 weeks of the study treatment period individuals in the Salba group consumed 38.5±7.6g/day (mean±SEM) and individuals in the control group consumed 39.5±10.7g/day. For the second 12 weeks of the study individuals in the Salba and control groups consumed 29.4±2.2g/day and 34.5± 5.5g/day, respectively. Supplement compliance for the entire treatment period was 81.9±4% for the Salba group and 73.4±5.9% for the control group (Table 5-9).

Table 5-9. Supplement prescription and intake at middle and end of treatment, n=20. Results are presented as mean±SEM.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>Control (n=10)</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0-12</td>
<td>Week 12-24</td>
<td>Week 0-24</td>
<td>Week 0-12</td>
</tr>
<tr>
<td>Prescribed Amount (g/d)</td>
<td>42.0±6.1</td>
<td>42.0±6.1</td>
<td>42.0±6.1</td>
<td>NS</td>
</tr>
<tr>
<td>Consumed Amount (g/d)</td>
<td>38.5±7.6</td>
<td>29.4±2.2</td>
<td>33.9±6.0</td>
<td>NS</td>
</tr>
</tbody>
</table>
5.3.5.2 Diet Analysis

Analysis of 3-day food records, depicted in Table 5-10, revealed that there were no significant changes in total caloric, carbohydrate, protein or fat intake from baseline to treatment-end in both treatment groups. Fibre intake was significantly increased from baseline in the Salba group at week 12 (p=0.018) and week 24 (p=0.021) and in the control group at week 12 (p=0.032) and week 24 (p=0.040).

Table 5-10. Comparing diet composition between treatment groups at baseline, middle, and end of treatment, n=20. Results are presented as mean±SEM.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>P-value</th>
<th>Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
<td>Week 0</td>
</tr>
<tr>
<td>Total Caloric Intake (kcal)</td>
<td>1625±171</td>
<td>1601±143</td>
<td>1619±140</td>
<td>NS</td>
</tr>
<tr>
<td>Carbohydrate Intake (g)</td>
<td>196±19</td>
<td>190±20</td>
<td>192±17</td>
<td>NS</td>
</tr>
<tr>
<td>Fibre Intake (g)</td>
<td>21±2</td>
<td>32±4</td>
<td>33±4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Protein Intake (g)</td>
<td>69±8</td>
<td>79±7</td>
<td>77±8</td>
<td>NS</td>
</tr>
<tr>
<td>Fat Intake (g)</td>
<td>65±10</td>
<td>63±7</td>
<td>70±8</td>
<td>NS</td>
</tr>
</tbody>
</table>

As described in Chapter 4, subjects were instructed to follow a hypocaloric diet of -25% of their individual daily energy requirements (DER) and then add to this diet their prescribed supplement amount. For individuals in the Salba group, this meant consuming 1550±72kcal daily, which was a 76±188kcal reduction from their baseline diet, as determined from the 3-day food records.
gathered at week 0. Individuals in the control group were instructed to consume a 1564±91kcal daily diet, which was a decrease of 440±274kcal from their baseline dietary intake. The total caloric intake of individuals in both groups is depicted in Table 5-11. Individuals in the Salba treatment group reduced their daily caloric intake from baseline by 25±156kcal and 6±147kcal, at weeks 12 and 24, respectively. This resulted in an intake of 105±11% of their prescribed calories at week 12 and an intake of 106±10% of their prescribed calories at week 24. Individuals in the control group reduced their total caloric intake from baseline by 154±302kcal at week 12 and increased it by 36±328kcal at week 24. This resulted in an intake of 121±13% of their prescribed calories at week 12 and an intake of 132±14% of their prescribed calories at week 24.

Table 5-11. Caloric prescription and intake at middle and end of treatment, n=20. Results are presented as mean±SEM.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=10)</th>
<th>P-value</th>
<th>Control (n=10)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 12</td>
<td>Week 24</td>
<td>Week 12</td>
<td>Week 24</td>
</tr>
<tr>
<td>Prescribed Amount (kcal)</td>
<td>1550±72</td>
<td>1550±72</td>
<td>1564±91</td>
<td>1564±91</td>
</tr>
<tr>
<td>Consumed Amount (kcal)</td>
<td>1601±143</td>
<td>1619±140</td>
<td>1850±175</td>
<td>2039±224</td>
</tr>
</tbody>
</table>
Chapter 6
Discussion and Conclusions

6.1 An Overview of the Results

Consumption of 33.9±6.0g/day of Salba appears to be safe, but did not demonstrate a significant effect on weight loss or related obesity or cardiovascular disease risk factors, when compared to control, after 24 weeks of treatment.

6.1.1 Effects on Weight

Analyses of the results gathered from this study indicate that Salba has no significant effect on weight loss, as compared to an oat bran control. After 24 weeks of supplementation with 33.9±6.0g of Salba, subjects’ weight increased by 0.47±1.16% of their total body weight, which was equivalent to an average weight gain of 0.35±0.85kg. Individuals in the control group had an average weight gain of 0.05±0.5kg. It can therefore be concluded that individuals in both the Salba and control groups did not achieve clinically relevant weight loss, but rather maintained their weight over the 24 week study period.

A modest amount of steady weight loss, about 5-10% of initial body weight, has been demonstrated to improve overall health outcomes (42). Specifically, sustained weight loss of ≥3.5kg can reduce the onset rate of diabetes by up to 58% (139). However, dietary interventions for weight loss have often proved unsuccessful. In a 2004 systematic review, it was concluded that the evidence for the use of dietary supplements as weight loss aids remains inconclusive (140). More specifically, the effect of fibre-rich dietary supplements has not shown consistent effects on body weight regulation or modification of additional health outcomes. In a double-blind placebo controlled study obese individuals with T2DM were administered 5g of psyllium 3
times daily, as an adjunct to dietary therapy, for a 6 week period. There were no significant changes observed in the participant’s weight, although fasting plasma glucose, TC, LDL and TG all showed significant reductions following the psyllium treatment (141). In another long-term study conducted by Pasman et al. (1997), 31 obese women were randomized to receive either 20g daily of a guar gum supplement or a control supplement for 14 months, directly following a 2 month energy-restricted period. No effect of fibre supplementation on weight, or related CVD risk factors, including blood pressure and cholesterol, was found (142).

The results of the present preliminary study are consistent with the results of a previous study where consumption of 50g/day of Chia seed, the original variety of Salvia hispanica L., for 12 weeks had no effect on weight loss (131). Despite the hypothesis that increasing intake of fibre, ALA, and protein, through the consumption of Salba, would induce significant weight loss, data from this study showed no differences in body mass between the Salba and control group after 24 weeks.

### 6.1.2 Effects on Glycemic Parameters

Analysis indicated that there were no significant end-differences in mean HbA1c, fasting glucose or fasting insulin for those in the Salba treatment group, compared to those in the control group. Although not shown to be statistically significant, after 12 weeks of supplementation with 38.5±7.6g/day Salba there was a 0.28% decrease in HbA1c. At week 12 there was also a decrease of 0.32mmol/L in fasting glucose and a decrease of 6.8pmol/L in fasting insulin from baseline, which represent a decrease of 5% and 9%, respectively. Although insignificant, these results are similar to those observed in the 12 week long RCT conducted by Vuksan et al. (2007), where individuals consumed 37g/day of Salba (130). Following 24 weeks of supplementation, however, when the mean intake of Salba decreased to 33.9±6.0g/day, there were no
improvements observed in HbA1c, fasting glucose or insulin values. Despite the lack of statistical significance, these results suggest that there may be a critical dose of Salba that must be consumed in order to have any measurable effect on glycemic control.

The moderate glycemic-lowering effect observed with Salba intake at week 12 may be attributed to the unique composition of Salba, which, as described in Chapter 2, was initially hypothesized to augment satiety, and consequently promote weight loss. As previously mentioned, Salba contains 35.2% dietary fibre. Dietary fibre has been shown to lower glycemia by a variety of mechanisms including: slowing the rate of digestion of starchy polysaccharides in the stomach, slowing the rate at which the stomach contents pass 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 reducing the rate of monosaccharide absorption through the microvilli of the intestinal epithelial cells (73,143).

While the physicochemical properties of different fibres have been used to explain their differing physiological responses on appetite and food intake regulation (72), they may also explain the differing effects of specific fibres on glycemic control. Both soluble and insoluble fibre have been shown to have beneficial effects on glycemia, however, soluble fibre has been shown to have more pronounced effects (144,145). Although only 16% of the total dietary fibre present in Salba is soluble, it is of a very high viscosity. Viscosity, defined as a liquid’s resistance to flow, is one of the most important rheological properties of fibre, as it is positively correlated with fibre’s ability to improve glycemic control (146,147). When viscous dietary fibre is exposed to water and human digesta it forms a gelatinous matrix and expands. This viscous mucilage prolongs the absorption of carbohydrates by slowing gastric emptying, and consequently slows the release of glucose into the bloodstream (148).
Consumption of fat and protein have also been demonstrated to delay gastric emptying, by stimulating gut hormones, such as GLP-1, leading some to suggest that this physiological effect comes as a result of the ingestion of all nutrients, and is not a characteristic distinct to fibre ingestion (81). However, literature reporting on the effects of polyunsaturated fat, the fat most abundant in Salba, on glycemic parameters remains highly inconsistent. In a review on the effect of n-3s on insulin resistance, the authors concluded that n-3 intake reduced insulin resistance in some, but not all, clinical studies (149). They also reported that while moderate amounts of n-3s (1-2g/d) did not effect glucose control in individuals with T2DM, intake of relatively high doses had an adverse effect on glucose homeostasis (149). In an analysis of 24-hr recall dietary intake assessments from 1284 individuals, it was determined that there was no significant association between polyunsaturated fatty acid intake and glycemic control (150).

The effect of protein on glycemic control also remains inconclusive. Attributing to the varying rates of digestion and absorption of individual amino acids, it is theorized that glycemic responses differ depending on the type of protein consumed (81). The ability of protein to improve glycemia has been attributed to delayed gastric emptying as well as to the promotion of insulin secretion by amino acids (151). In a study by Gannon et al. (1988), the plasma glucose and insulin responses to glucose consumed alone or with lean beef, turkey, gelatin, egg white, cottage cheese, fish or soy was examined in individuals with T2DM. Compared to the glucose alone, all meals, with the exception of egg white, significantly decreased glucose AUC and increased insulin AUC (152). However, the ingestion of protein has also been suggested to increase glycemic responses, reportedly by stimulating glucagon secretion, converting amino acids to glucose, and increasing insulin resistance (153).
6.1.3 Effects on Obesity-Related Outcome Measures

In accordance with the guidelines set out by the WHO, in this study BMI was used as a clinical indicator of overweight (BMI $\geq 25$ kg/m$^2$) and obesity (BMI $\geq 30$ kg/m$^2$) (5). However, BMI has received a lot of criticism in recent years due to its inability to account for different ethnicities (154). Furthermore, BMI does not take into consideration the breakdown of fat and lean muscle mass or the distribution of body fat, which may be a better predictor of T2DM and CVD risk (155). Consequently, in this study, additional measures of body composition, including percent body fat (%BF) and waist circumference (WC), were carried out in order to gain further indication into the participants’ health status.

Examination of %BF using BIA revealed that after 24 weeks the %BF of individuals in the Salba and control groups increased from baseline by 0.72±0.94% and 0.98±0.61%, respectively. There was no significant change in %BF in either of the treatment groups from baseline to the end of the treatment period. However, after only 12 weeks of supplementation with Salba, individual’s %BF decreased by 0.22±0.39%. This was not significant when compared to baseline, however, compared to control, nearly approached statistical significance (p=0.057).

When measured using DXA, following 24 weeks of supplementation, individuals in the Salba treatment group had a decrease in %BF of 1.4±1.2%, whereas the %BF of individuals in the control group increased by 0.1±0.7%. However, no significant differences were detected between the two treatment arms or within either of the treatment groups.

Waist circumference (WC), or the ratio of waist to hip circumference (WHR), is another method that is often used as an estimate of body fat distribution and an indicator of obesity. Following 12 weeks of supplementation with Salba, individual’s WC decreased by 1.7±1.4cm, whereas the
WC of individuals in the control group decreased by 1.6±0.7cm. In the subsequent 12 weeks, the WC of individuals in the Salba group continued to decrease by an additional 0.7±0.6cm, resulting in a total change of 2.4±1.6cm from baseline. After 24 weeks the WC of the control group increased by 0.15±0.3cm from baseline. No statistically significant differences were founds between either treatment group or within the treatment groups.

Percent body fat and waist circumference have both been strongly associated with hypertension, dyslipidemia, coronary heart disease and T2DM (156,157). Specifically, elevated central, or abdominal, obesity has been repeatedly linked to increased risk of developing T2DM (158). In a study of 21 men with T2DM, visceral adipose tissue accumulation was shown to be strongly correlated with insulin sensitivity (159). Furthermore, in a prospective cohort study of 27,270 men central adiposity, as determined by WC, was shown to predict the risk of T2DM after 13 yrs follow up, independently of overall obesity assessed by BMI (160).

Men with a WC ≥102 cm and women with a WC ≥88cm are at increased risk of metabolic complications (1) and, consequently, these values have been set as the cut-off points recommended for healthy individuals (160). In Caucasian populations, the %BF cut-off point for obesity is 25% in males and 35% in females (161). However, although fairly strong correlation between BMI and %BF has been demonstrated in Caucasian populations (162), this relationship cannot be applied to other populations (163). As a result, and due to the high variability in %BF depending on a individual’s age and sex, specific %BF cutoff points for the general public have not been set (161).

Therefore, despite no significant reductions in weight, the decreasing trend in both %BF and WC observed in the Salba treatment group, may represent promising clinical findings that support the use of Salba as an adjunct dietary treatment option for obesity.
6.1.4 Effects on Cardiovascular Disease Risk Factors

Analysis of the results revealed that dietary supplementation with Salba for 24 weeks has no effect on additional CVD risk factors, including blood pressure, lipids and inflammation.

From baseline, the office systolic blood pressure of individuals in the Salba and control groups increased by 4.3mmHg and 6.6mmHg, respectively. Diastolic blood pressure also increased by 1.8mmHg in the Salba group and 0.9mmHg in the control group. However, statistical analysis revealed that no significant differences in either group were achieved.

In a meta-analysis of 31 placebo-controlled trials the authors concluded that there is a dose-response effect of intake of n-3s from fish oils on BP (164). Furthermore, in a randomized crossover study, assessing the cardiovascular responses of hypercholesterolemic subjects, participants who were fed an ALA-rich diet demonstrated a significant reduction in DBP (165). It is hypothesized that the reduction in BP occurs through the conversion of ALA into EPA, which results in the modification of the eicosanoid pathway and a subsequent decrease in the production of vasoconstrictive prostaglandins (164). Conversely, in studies on the effects of fibre intake and BP a clear relationship has not been demonstrated. In a RCT of 88 normotensive individuals, no changes in SBP or DBP were observed after consumption of a high fibre diet for 6 weeks (166).

The lack of a significant effect of Salba on BP, as demonstrated in the present study, is in contrast to the results of a previous study, where Salba was shown to significantly decrease SBP after 12 weeks of supplementation (130). However, the results of this study may be explained by the already optimal baseline BP of the study population, which can be attributed to the antihypertensive medication taken by the majority of subjects, as indicated in Chapter 5.
In this study no significant changes in serum total cholesterol, high-density lipoprotein, low-density lipoprotein or triglycerides were observed in either treatment group.

The relationship between increased fibre intake and improved lipid profiles has been well-documented. Specifically, viscous fibres have been shown to be positively correlated with reduced LDL. In a randomized crossover trial on 23 participants fed fibres of different viscosities, LDL reduction was shown to be greater in high viscosity fibres, compared to lower viscosity fibres, despite a smaller quantity consumed (167).

In the literature, dietary n-3 supplementation has been demonstrated to reduce serum triglycerides in individuals with T2DM, but may raise LDL levels (168). In the Lyon Diet Heart Study, the addition of n-3 to a high-carbohydrate low-fat Mediterranean diet did not have any effect on TG, LDL, HDL, or TC. However, there was a 65% reduction in CHD mortality. These findings suggest that changes in traditional risk factors, such as blood lipids, are not the sole cause of CHD (169). In the present study, supplementation of Salba, which contains 19.8% n-3s, showed no detrimental effect on blood lipid profile in individuals with T2DM, who are typically more susceptible to heart disease than the nondiabetic population.

Inflammation plays a major role in obesity and CVD and therefore measurement of inflammatory markers, such as hs-CRP may be beneficial for overall risk assessment (170). However, many previous prospective studies evaluating the effects of either fish oil or ALA have not found significant changes in hs-CRP levels with increased n-3 consumption (171-173). In an epidemiological study examining the effect of dietary fibre intake on hs-CRP, the authors concluded that, in the presence of weight loss and improved dietary fat intake, increased fibre consumption is significantly correlated with lower concentrations of hs-CRP (174).
In this study there was no significant difference in hs-CRP between the Salba and control groups at any of the time points examined. However, there was a trend observed in the Salba group, where hs-CRP decreased from 3.3±0.8 mg/L to 3.1±0.8 mg/L after 12 weeks of supplementation with Salba, and decreased even further to 2.98±0.9 mg/L after 24 weeks of supplementation. This trend is similar to that observed in a previous long-term study, where hs-CRP decreased by 7.0±2.3% from 3.1±2.4 mg/L to 2.9±2.3 mg/L after 12 weeks of supplementation with 37g/day of Salba (130).

6.2 Study Limitations

There are several limitations of this study, which must be considered when interpreting these preliminary results.

6.2.1 Sample Size

Power analysis revealed that in order to observe a significant effect on the primary outcome, weight, 132 subjects needed to be included in this study. However, due to slow study recruitment and a greater than expected attrition rate, at the time of data analysis only 21 subjects had completed the study, one of which was not considered in the analysis due to low supplement compliance. Based on the promising preliminary findings from the 20 subjects included in this analysis, this study will continue to be carried out.

6.2.2 Data Analysis

The baseline differences in subject characteristics between the two treatment groups served as a limitation in this investigation. Comparison of baseline characteristics revealed that individuals
in the Salba treatment group had a lower BMI, compared to the control group. An attempt was made to consider this variation in the analysis of the results.

6.2.3 Study Population

Participants in this study had stable, well-controlled T2DM (HbA1c 7.1±0.2% [mean±SEM]) and many were already taking some of the most efficacious medical therapies currently available, which were kept unchanged throughout the entire treatment period of the study. Therefore they may not represent typical candidates for adjunctive therapy. The nonsignificant results observed in this study may be attributed to the already optimal baseline glycemic control achieved by the subjects’ underlying diabetes therapy.

6.2.4 Study Compliance

One of the major challenges in research examining long-term dietary supplementation for weight loss is ensuring subjects’ compliance to the study protocol (140). All subjects included in the analysis of this study consumed >50% of prescribed supplements. Supplement compliance was not significantly different in either treatment group. However, it was greater at week 12, compared to week 24, in both the Salba group (93.5±6% vs. 81.9±4%) and the control group (78.8±5.2% vs. 73.4±5.9%).

The taste of Salba is considered to be fairly neutral; and in a previous long-term trial only 40% of individuals randomized to consume Chia seeds positively identified that they were consuming Chia seeds, as oppose to a placebo supplement (131). However, Salba is becoming increasingly available in many grocery and health food stores. This may have played a role in the study blinding, as individuals may have been aware of what treatment they were on, although this was
not directly asked of the participants of this study. This may serve as a partial explanation as to why supplement compliance was greater in the Salba group than in the control group.

In addition to being prescribed 42.0±6.1g/day of Salba or 50.1±9.2g/day of the control supplement, participants were also instructed to reduce their caloric intake by -25% of their DER. This translated to a mean recommended decrease of only 76±188kcal/day from baseline for individuals in the Salba treatment group. However, current clinical guidelines recommend a reduction in energy intake by 500-1,000 kcal/day in order to achieve measurable weight loss (7). Furthermore, despite the recommended decrease of 76±188kcal/day, individuals in the Salba group only reduced their energy intake from baseline by 25±156kcal/day and 6±147kcal/day, at weeks 12 and 24, respectively. Individuals in the control group also consumed more calories than recommended for the duration of the study.

Analysis of the 3-day diet records revealed that individuals in both treatment groups consumed more calories at week 24 than they did at week 12. This may have been due to the lack of rigorous follow-up. Individuals attended the clinic for follow-up visits more frequently in the first 12 weeks of the study, compared to the latter 12 weeks. In a study examining the factors associated with dropout from a 6-month long weight loss intervention trial, the authors concluded that the rate of weight loss during the initial weeks of an intervention is integral to ensuring completion of the study (175). With this in mind, the study protocol was designed in order to maximize support for study participants during the initial weeks of the study, when they would be adjusting to the intervention.

Subjects were instructed to follow a macronutrient diet profile in adherence with the 2008 Canadian Diabetes Diet Guidelines: 45-60% of energy as carbohydrates, 15-20% of energy as protein, and <35% of energy as fat. Analysis of the 3-day food records revealed that subjects in
both the Salba and control groups were already consuming a diet that followed these
commendations at baseline. Analysis of the 3-day food records at the middle and end of the
study treatment period revealed that individuals in both the Salba and control groups maintained
their dietary macronutrient profile throughout the study period.

Although recent statistics indicate that the average North American consumes less than 15g of
dietary fibre per day (73), the baseline fibre intake of individuals who participated in this study
was 22.9±1.8g/day, which is within the recommended intake of 21-38g/day (70). Subjects were
prescribed supplements of Salba or control, which were matched for total dietary fibre, that
contained a mean of 14.7g of fibre. Analysis of the 3-day diet records obtained at the end of the
study period revealed that after supplementation with either Salba or control supplement, fibre
intake increased by 11.9±3.0g to 34.8±3.1g/day. Further proof of compliance could be supported
by analysis of the plasma total fatty acid composition, specifically ALA, of subjects at the end of
treatment, as was done in a previous Salba RCT (130). However, at present time, the samples
have not been analyzed for ALA.

Non-compliance to the study diet may be attributed to the passive nature of the dietary
intervention. The nutritional intervention program used was based on CDA’s Beyond the Basics:
Meal Planning for Healthy Eating, Diabetes Prevention and Management. This was selected in
order to provide a healthy and balanced dietary approach that could be adapted to each of the
study participant’s individualized needs. However, this nutritional education intervention did not
demonstrate a significant effect on any of the outcome measures in either treatment group. In
previous weight loss studies using similar lifestyle recommendation approaches, poor subject
compliance has been cited as a result of lack of resources, lack of practitioners’ time or skill, or
lack of subject motivation (139), all of which may have significantly affected the outcomes of this study.

Poor adherence to weight loss study interventions have also been documented as a result of overly complicated study protocols (176). It has been demonstrated, that single behaviour interventions, targeting either diet or physical activity, may be more effective than multiple health behaviour interventions, which target both diet and physical activity (177). In a recent study involving 280 women, different physical activity interventions were examined. The authors reported that the intervention which included both recommendations on specific diet and physical activity modifications did not significantly change the physical activity levels or the dietary behaviours of the women, compared to those women who received only a physical activity intervention (178). This supports the hypothesis that changing multiple behaviours simultaneously may be overwhelming for individuals and, consequently, decrease the effectiveness of multiple health behaviour interventions (177). Subjects in this study were instructed to modify their diets, but to maintain their current level of physical activity. Consequently, physical activity during this study was not quantified; however, it may be an important factor to consider when interpreting the study results.

6.2.5 Control

Contrary to the previous long-term study carried out on Salba (130), which compared Salba to a wheat bran control, in this study a blend of oat bran, inulin and maltodextrin was selected as the control. Oat bran has been demonstrated to have beneficial effects on cholesterol (179). More specifically, the main component of oat soluble fibre, β-glucan, has been shown to reduce LDL cholesterol and consequently reduce the risk of heart disease (180). In this study oat bran was
used as a positive control. However, because of this the effect of Salba on cholesterol, and potentially other factors, may have been obscured.

### 6.2.6 Methodology

There are several caveats with the methodology used to collect some of the measurements that were used in this study. First, it is evident that there is some variation between the two methods used to determine %BF, namely BIA and DXA. At baseline, measurements done by BIA were $5.5\pm1.0\%$ lower than those gathered using BIA and at week 24 they were $3.98\pm0.9\%$ lower. Although the technical error associated with BIA and DXA are relatively low, $<2\%$ and $<3\%$, respectively (161), several factors may play a role in influencing the measurements produced. BIA measurements are dependent on body temperature, posture, and body shape (161). Furthermore, water distribution between the intra and extracellular spaces may serve as an additional source of error, as the different compartments have different specific resistivity (161). DXA, which uses a three-compartment model to assess body fat, is reliant on the accurate estimation of hydration of fat free mass (181) and tissue depth (161).

Another source of methodological error could have arisen from the waist and hip circumference measurements. Although all anthropometric measurements were carried out by the same individual for each subject who participated in this study, WC and HC measurements have been shown to have relatively high measurement error and ethnical bias (182). Furthermore, WC measurements have been demonstrated to have even greater variation in males with higher BMIs (182).

In this study, subjects’ diets were assessed using 3-day food records that were completed prior to each study visit. However, as with other diet assessment techniques, such as food frequency
questionnaires and 24hr recalls, dietary food records have been reported to give unreliable estimates of actual food intake. When compared to direct observation, both overestimation and underestimation of food intake has been observed with food records (183). Furthermore, food records may not be representative of usual food intake, as the need to record intake may lead to a more homogenous and simplified diet. Another issue that arises with food records is compliance, as recording such detailed information can become tedious. Consequently, important information regarding the type and amount of food consumed is often omitted and subsequent analysis of the record relies on deductions made by the study researcher.

### 6.3 Future Directions

The results presented here provide rationale for the continuation of this study. The consumption of 33.9±6.0g/day of Salba or 37.0±11.5g/day of the control supplement appears to be safe, as no adverse events were reported. As indicated in Chapter 4, in order to detect significant differences in weight loss, 54 participants per group are required. Therefore, although there were no significant effects of Salba or control on any of the outcome measures in this population, (n=20) continuation of the study may determine otherwise.

The mean weight change for individuals enrolled for 24 weeks in this study was 0.2±0.5kg. It has been estimated that body weight increases by 0.5kg/year in the general population (184), and therefore even maintaining one’s weight may be useful for disease prevention. Furthermore, it may be beneficial to explore the potential use of Salba for weight maintenance in people who have already achieved their weight loss goals. In a RCT, individuals with the highest success rates in attaining their lifestyle objectives, which included >4 hrs/wk of moderate physical activity, <30% total energy intake from fat, and 15g of fibre/1000kcal daily, were those who already met the objectives at baseline (185). Although supplement compliance was considerably
high in this study, future long-term studies may consider administering the treatment supplements in different formats, such as breads or bars, in order to add variation to the subjects’ diets.

In a design such as the one used in this study, no specific functional component from Salba can clearly be implicated in any of the potential health benefits seen. Therefore, if the continuation of this study reveals significant results in any of the outcome measures, additional mechanistic studies will be required. Only the total energy and total dietary fibre of the Salba and control treatments were matched in this study. Future studies may attempt to match the content of other dietary components, such as protein or polyunsaturated fat. Furthermore, specific components of the grain could be removed in order to elucidate the specific nutrients responsible for the proposed health effects of Salba. In addition, analysis of regulatory gut hormones, such as GLP-1, adiponectin and ghrelin, could be valuable to determine the metabolic effects of Salba and possibly elucidate the mechanisms by which it acts.

6.4 Conclusions

The hypothesis that dietary supplementation of Salba would assist with weight loss in individuals who are overweight or obese and have T2DM is not supported by the preliminary findings presented here. However, the results did demonstrate an insignificant improvement in %BF and WC after 12 weeks of supplementation with Salba. Similarly, findings of the current study do not support the hypothesis that Salba has a positive effect on glycemic control, but after 12 weeks there was a trend towards improved HbA1c, fasting glucose and insulin. Several caveats and sources of variability exist in the presented research. Most importantly, an additional 88 subjects must complete the study in order to achieve sufficient power to conduct appropriate statistical analysis and draw valid conclusions. The findings of this study support the hypothesis that Salba
would not affect safety parameters, which is consistent with previous literature and provides further safety evidence for Salba use. Given the high prevalence of obesity and T2DM, further investigation into Salba’s potential to act as a novel dietary therapy for weight loss and additional CVD and T2DM risk factors is therefore warranted.
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Appendix 1: Telephone Screening Questionnaire

**TELEPHONE SCREENING QUESTIONNAIRE**

If the individual does not meet the inclusion criteria for the study, please thank them for their interest in the study and explain that the protocol must follow strict inclusion and exclusion criteria because of the specific research question we are interested in.

1. Have you been diagnosed by a doctor with type 2 diabetes?  Must answer YES

2. Are you overweight?  Must answer YES. What is your height ________ weight________
   Calculate BMI (=w/h²) ________ (must be 25-40 Kg/m²)

3. Are you currently taking any hypoglycemic agents, herbal remedies or supplements of any kind?  If YES, please list:
   a. __________
   b. __________
   c. __________
   d. __________
   e. __________
   f. __________

   *** MAY NOT INCLUDE those with recent changes in prescribed medications that may affect weight, including antidepressants, glucocorticoids, diuretics, laxatives, Xenical (orlistat), Meridia (sibutramine), fish oils, or other investigational weight-loss drugs or

4. Have you been diagnosed with depression?  Must answer NO

5. Are you between the ages of 35-75?  Must answer YES

6. Do you have any kidney or liver problems?  Must answer NO

7. Are you pregnant?  Must answer NO

8. Do you have any other major illnesses or gastrointestinal problems (eg: Irritable Bowel Syndrome, Crohn’s disease, Colitis)?  Must answer NO.

9. Do you have high blood pressure?  May answer YES (if on medications, okay, except for recent changes in diuretic medications.  If it is significant (SBP >160mmHg, DBP>100mmHg) on multiple readings, exclude them; if borderline (SBP 140-159 mmHg, DBP 80-100 mmHg), then include).

10. Do you consume > 2 alcoholic drinks per day?  Must answer NO

11. Do you regularly smoke tobacco or marijuana, or use other smokeless nicotine products?  Must answer NO

12. Are you on insulin therapy?  Must answer NO
13. Do you use any laxatives? Including bulk-forming laxatives? Must answer NO

14. Do you take any high fibre supplements such as flax seed, bran, Benefibre, Guar gum. Must answer NO

15. Do you have cancer (must answer NO) unless superficial (i.e. skin). Are you on Cancer therapeutic agents (must answer NO).

16. Do you have unstable angina, or have you had a M.I. or stroke within the previous 6 months? Must answer NO

17. Have you had a significant weight change within the previous 3 months? If yes, how much weight gained or lost (in kg) If YES, must be less than 10% of total body weight

18. Have you been actively dieting within the last month to lose weight? If YES – under discretion of interviewer (i.e. if they have lost 1 pound in the last month then they may be included but if they have lost more than 5 pounds exclude them).

19. Do you currently have an eating disorder (anorexia or bulimia)? Must answer NO.

20. Are you able to give blood samples? Must answer YES

21. Are you able to come to the clinic for 6+ separate appointments that will begin between 7:30am and 9:30 am and take between 0.5 and 4 hours? Must answer YES.

22. Are you able to arrive at these visits in a fasted state (i.e. having not eaten or consumed any liquid within 10-12 hours prior to arriving at the clinic? Must answer YES.
Appendix 2: Informed Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF RESEARCH STUDY

Efficacy and Safety of Whole Grain Salba (Salvia Hispanica L.) on Weight Loss in Overweight and Obese Individuals with Type 2 Diabetes

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STATEMENT OF CONSENT

TITLE OF RESEARCH STUDY

**Efficacy and Safety of Whole Grain Salba (Salvia Hispanica L.) on Weight Loss in Overweight and Obese Individuals with Type 2 Diabetes**

CONSENT

I acknowledge that I have been given sufficient time to read and understand the preceding, the research study described there-in has been explained adequately, and any questions that I had have been answered to my satisfaction. I certify that I have been informed of (1) the procedures I will follow; (2) the potential risks, harms, and discomforts that may result from these; (3) compensation I will receive, should I choose to participate; (4) assurance that records relating to my involvement will be kept confidential and information will not be released without my permission unless required by law; (5) the possibility of publication or presentation of the results of this study and the means that will be taken to ensure confidentiality; and (6) alternatives to participation in this study, including the right not to participate and withdraw without compromising the quality of medical care at St. Michael’s Hospital for me or the other members of my family. If I have any further questions regarding these matters, then I know that I may ask them now or in the future.

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

Would you like your family doctor to be informed of your results?   Yes___   No ___

Participant name:__________________   Participant signature:_________________

Date:__________

Name and position of the person obtaining consent:

________________________________________________________

Signature of position of person obtaining consent: ___________________________

Date: _________
Appendix 3: Medical Information Form

MEDICAL INFORMATION FORM

All information provided in this questionnaire will be kept confidential and released only for the purpose 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: ________________________________

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:_______ mmol/L
Post-meal glucose:_______ mmol/L
HbA1c (glycosolated haemoglobin)_____%
Rx:________________________
________________________________________
Complications:________________
________________________________________

When:__________  How high: 
sBP/dBP:_______/_______ mmHg
Rx:________________________
________________________________________
Complications:________________
________________________________________

Office use only:

Ht (cm): Wt (kg): BMI:
Waist: Hip Ratio:
Waist Circumference (cm):
Blood pressure (mmHg):
% Body Fat:
Have you been diagnosed with any of the following? (If yes, please indicate onset date, treatment and current status- recovered/ active condition)

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>NO</th>
<th>YES</th>
<th>Onset date</th>
<th>Present status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Recovered</td>
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<td></td>
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<td></td>
<td>Active</td>
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<tr>
<td></td>
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<td></td>
<td>(please indicate treatment)</td>
</tr>
<tr>
<td>Malabsorption syndrome</td>
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<tr>
<td>Crohn’s</td>
<td></td>
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<tr>
<td>Ulcerative colitis</td>
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<tr>
<td>Stomach (gastric) ulcer</td>
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<tr>
<td>Duodenal ulcer</td>
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<tr>
<td>Intestinal parasites</td>
<td></td>
<td></td>
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<tr>
<td>Diarrhea (&gt; 2 liquid stools/day)</td>
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<tr>
<td>Constipation (≥ 3 days duration)</td>
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<tr>
<td>Anorexia or Bulimia</td>
<td></td>
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<tr>
<td>Heart disease</td>
<td></td>
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<tr>
<td>Stroke</td>
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<tr>
<td>Heart attack</td>
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</tbody>
</table>

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

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)
### Arrhythmia

### Uncontrolled hypertension
- Systolic BP ≥ 140
- Diastolic BP ≥ 90

### Blood clotting disorders

### Anaemia

### Kidney disease

### Psychiatric conditions (i.e Depression)

<table>
<thead>
<tr>
<th>CONDITION</th>
<th>No</th>
<th>Yes</th>
<th>Onset date</th>
<th>Present status</th>
<th>Active (please indicate treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious hepatitis (B, C, D)</td>
<td></td>
<td></td>
<td></td>
<td>Recovered</td>
<td></td>
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<tr>
<td>Recently diagnosed infectious hepatitis A, E</td>
<td></td>
<td></td>
<td></td>
<td>Recovered</td>
<td></td>
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<tr>
<td>HIV/ AIDS</td>
<td></td>
<td></td>
<td></td>
<td>Recovered</td>
<td></td>
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<tr>
<td>Tuberculosis</td>
<td></td>
<td></td>
<td></td>
<td>Recovered</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td>Recovered</td>
<td></td>
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<tr>
<td>Thyroid disease</td>
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<tr>
<td>Do you experience any of the following:</td>
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<tr>
<td>Fatigue</td>
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<td></td>
</tr>
<tr>
<td>Unexplained weight gain</td>
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<td></td>
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<tr>
<td>Dry skin and hair</td>
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<tr>
<td>Depressed mood</td>
<td></td>
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<tr>
<td>Cold intolerance</td>
<td></td>
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</tr>
<tr>
<td>Constipation</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Increased cholesterol?</td>
<td></td>
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<tr>
<td>Nervousness/irritability</td>
<td></td>
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<td></td>
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<tr>
<td>Palpitations</td>
<td>Heat intolerance</td>
<td>Increased sweating</td>
<td>Unexplained weight loss</td>
<td>Insomnia</td>
<td>Pancreatic disease</td>
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</tbody>
</table>

Any other health problems?  □ No  □ Yes (please describe)
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________
______________________________________________________________________________

**Lifestyle and diet**

Are you following a special diet?  □ No  □ Yes

If yes, please describe
______________________________________________________________________________

Have you ever been on a weight loss diet?  □ No  □ Yes

If yes, when was the last time you have been on a diet:
______________________________________________________________________________

How long did you stay on that diet?
______________________________________________________________________________

How many times have been on a weight loss diet?
______________________________________________________________________________

Which type of diet(s) have you tried following in the past? (e.g. general calorie restriction, eliminating certain foods/food groups, Atkins, Bernstein, South beach, Weight...
What was the maximum weight that you lost during a diet?

Who has encouraged you to go on a diet (check all that apply)

☐ Self  ☐ Family member/friend  ☐ Health care professional  ☐ Other: __________

What motivated you to lose weight?

☐ Health  ☐ Appearance  ☐ Major life event (please specify) __________
☐ Other: __________

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?

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

Have you participated in a clinical trial within the last 2 months? ☐ Yes ☐ No

Did you have blood sample drawn? ☐ Yes ☐ No

Did the nurses experience difficulty in drawing blood samples from you? ☐ Yes ☐ No:

If yes, what difficulty did the nurse encounter?

☐ Finding veins ☐ Problems of bleeding ☐ Other: ___________

Did you experience any discomfort during or after blood samples have been collected from you?

☐ No ☐ Yes

If yes, please describe: ☐ Nausea ☐ Fainting ☐ Dizziness ☐ Other: ________
Appendix 4: Dietary Questionnaire

Nutrition and Lifestyle Questionnaire

FOOD / EATING HABITS (please check all that applies)

- How often do you: Eat Out: ______________; Grab and Go: ___________; Order In: ______________
- How many people in your home? ______________
  Support System: □ Family □ Friends □ Other: __________
- Who’s in charge of the COOKING? ______________
  GROCERY SHOPPING? __________
- Which pattern of eating typifies your style?
  □ Regular meals at frequent intervals □ Occasionally skip a meal □ Skip Breakfast or Lunch
  □ Skip meals during the day and eat only the evening meal □ Snacking / grazing throughout day
- Describe changes, if any, that you made to your eating habits. When did you implement these changes?
  __________________________________________________________________________________________
  __________________________________________________________________________________________
  __________________________________________________________________________________________
- How many meals do you consume per day? □ One □ Two □ Three
  ▪ Which meal do you skip? __________
- Which meal is the LARGEST? □ Breakfast □ Lunch □ Dinner
  □ Snacks
- Do you use food for reward or escape? □ No □ Yes
  ▪ What foods/beverage, and how often? ____________________________
  ____________________________
  ____________________________
- What foods would be most difficult to give up?
  ____________________________
  ____________________________
  ____________________________
- Do you associate food consumption with any stressor? □ No □ Yes
  Stressor(s): __________________________
- Do you have specific food cravings? □ No □ Yes
  ▪ What foods? __________________________
  __________________________
  __________________________
- Which of the following might tempt you?
<table>
<thead>
<tr>
<th>Coffee break at work</th>
<th>Hunger</th>
<th>Watching TV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passing by fast food places when hungry</td>
<td>Stress, frustration, anger</td>
<td>Working late</td>
</tr>
<tr>
<td>Celebrating B-days, anniversaries, special events</td>
<td>Skipping meals</td>
<td>Boredom</td>
</tr>
<tr>
<td>Eating out with friends</td>
<td>Traveling, having to eat on road</td>
<td>Partying with friends</td>
</tr>
<tr>
<td>Sport games at arenas, movie theatres</td>
<td>Seeing a food advertisement</td>
<td>Other: ____________________</td>
</tr>
</tbody>
</table>

**MEAT AND ALTERNATIVE**

- How many servings of **MEAT, FISH, POULTRY** items do you consume per day? Please include all meals. (One serving = size of a deck of cards, about 3 ounces or 90 grams)
  - □ MORE than 2
  - □ Two
  - □ One
  - □ LESS than one

- How often a week do you eat **RED MEATS**? (Beef, Steak, Pork, Ribs, Bacon, Lamb)
  - □ More than 7 times
  - □ 5 to 6 times
  - □ 3 to 4 times
  - □ 2 times

- How often a week do you eat the following **Processed Meats**: Hot Dog, Bologna, Luncheon Meat, Bacon, Ham, Sausage, Meat Spreads?
  - □ 4 or more times
  - □ 3 to 4 times
  - □ 1 to 2 times
  - □ Rarely or Never

- How many servings of **MEAT ALTERNATIVES** (tofu, soy, dried legumes: peas, beans, lentils, etc…) do you consume per week? Please include all meals. (One serving = ½ cup or 3 ounces)
  - □ More than 7 times
  - □ 5 to 6 times
  - □ 3 to 4 times
  - □ 2 times

- How often a week do you eat **FISH**? _____________

- How often a week do you eat **EGGS**?
  - □ More than 7 times
  - □ 4 to 6 times
  - □ 2 to 3 times
  - □ Once or none

**GRAINS, BREADS, CEREALS**

- When choosing **BREADS and CEREALS**, do you most often choose:
  - □ Whole Grain breads, cereals
  - □ White bread only
  - □ Variety of Whole Wheat, Rye, White, etc…

- Do you include the following foods in your diet?
  - **SOLUBLE FIBRE sources**:
    - □ Oat, Oat bran
    - □ Barley
    - □ Apples, Pears, Berries, Citrus fruit
    - □ Lentils, dried Peas, Beans
    - □ Carrots, Peas, Sweet Potatoes
    - □ Chick peas
    - □ Flax
    - □ Psyllium
- Almonds  □ Soy products

**INSOLUBLE FIBRE sources:**
- Wheat Bran  □ Bulgur
- Whole Wheat Bread  □ Buckwheat
- Corn Bread  □ Brown Rice
- Whole Grain Cereal  □ Fruits and Vegetable with SKIN

How many servings of fibre sources (named above) do you have each week?
- MORE than 10  □ Every day  □ 3 to 5 times  □ 1 to 2 times  □ Not at all

- About how many times a week do you consume **COMMERCIAL BAKED PRODUCTS** (i.e., Donuts, Cookies, Muffins, Pastries, Tarts, Pies, etc…) each week?
- MORE than 10  □ Every day  □ 3 to 5 times  □ 1 to 2 times  □ Not at all

### FRUITS AND VEGETABLES

- How many servings of **FRUIT** do you consume each day? (1 servings = 1 medium fruit, ½ cup juice, ½ cup canned fruit)
  - 4 or more  □ Three  □ Two  □ One  □ None
- Do you consume **FRUIT JUICE**?
  - Yes  □ No
- How many servings of **VEGETABLES** do you consume each day? (1 servings = 1 cup mixed salad, 1 raw vegetables, ½ cup cooked vegetables)
  - 4 or more  □ Three  □ Two  □ One  □ None
- Which describes your consumption of vegetables?
  - Snack on raw vegetables and eat vegetables/salads with most meals
  - Eat salads and/or vegetables at one meal a day  □ Eat vegetables 2-3 times per week
  - Rarely eat vegetables

### DAIRY PRODUCTS

- Which type of **DAIRY PRODUCTS** (Milk, Yogurt, Ice-cream, Cheese) do you consume most frequently?
  - Regular  □ Homogenized  □ 2%  □ 1%  □ Skim  □ Not at all  □
  - Other: ________
- How much **MILK** or **YOGURT** do you consume per day? __________ cups per day
- About how many servings (1 ounce servings) of **HIGH FAT CHEESES** do you consume each week? (i.e., cheddar, swiss, brie, mozzarella, etc…)
  - MORE than 10  □ Once per day  □ 3 to 5  □ 1 to 2  □ None
  - Do you eat **LOW FAT CHEESES**?
  - Yes  □ No

### OTHER FOODS

- How many snacks do you consume a day?
- About how many times do you consume **HIGH FAT SNACK or SWEET** foods in a week? (i.e., chips-potato, corn, taco; nuts; ice-cream; desserts; sugar-based beverages; chocolate; etc…)
  - □ Every Day  □ 3 to 5 times per week  □ 1 to 2 times per week  □ Rarely or Never

- How often do you eat **HIGH FAT FAST FOOD** Meals? (hamburger with fries, poutine, hot dogs, etc…)
  - □ MORE than Once a week  □ Once a week  □ Once every 2 weeks  □ Once a month  □ Rarely

- Which method of cooking is used most frequently in your household?
  - □ Frying with Butter/Margarine/or Oil  □ Baking/Roasting  □ Broiling  □ Microwave  □ BBQ
  - □ Other ______

- Which of the following do you use more often at home?
  - □ Butter  □ Margarine  **Brand:**

- Please state the type of **COOKING OIL** you are presently using at home?
  - ______________________________________________

- Do you add **SALT** to your **Meals**?  □ Yes  □ No
  - **Cooking**  □ Yes  □ No

- In what form do you most frequently purchase food or meal preparations?
  - □ Fresh  □ Canned, Frozen **without** Salt  □ Canned **without** Sauces
  - □ Canned, Frozen, Dry with Sauces or Seasonings

- While preparing meals or when eating out, how frequently do you add any or all of the following items to your food? pickles, relish, soy sauce, ketchup, meat tenderizer, MSG?
  - □ Daily  □ 3 to 4 times per week  □ 1 to 2 times per week  □ Rarely or Never

- How do you have your coffee, tea or cereal?  □ Sugar  □ Artificial Sweetener  □ Creamer  □ Milk
  - □ Black

- How many drinks containing **ALCOHOL** do you consume each day? (1 serving = 5oz wine, 12 oz beer, 1.5oz shot)
  - □ MORE than ONE per day - How many? _______  □ One  □ LESS than one  □ None

- How many glasses of **WATER** do you drink in a day?
  - □ 8 or more glasses  □ 5 to 8 glasses  □ 2 to 4 glasses  □ One glass or none

- How much **TOTAL FLUID** do you consume a day (Water, Juice, Coffee, Tea, Milk)?
  - ______________________________________________
Appendix 5: Physical Activity Questionnaire

Habitual Physical Activity Questionnaire

Please answer the following questions by circling which value best applies to you and by filling out all questions that require a written response.

<table>
<thead>
<tr>
<th>1- Never</th>
<th>2- Seldom</th>
<th>3- Sometimes</th>
<th>4- Often</th>
<th>5- Very</th>
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</thead>
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</tbody>
</table>

1. What is your main occupation? __________________________________________


5. At work I lift heavy loads… [1] [2] [3] [4] [5]


8. In comparison with others my own age I think my work is physically demanding… [1] [2] [3] [4] [5]

9. Do you play sports? YES / NO

If YES;
- Which sport do you play most frequently? __________________________


If you play a second sport;
- Which sport is it? ____________________________


15. In comparison with others my own age I think my physical activity during leisure time is… [1] [2] [3] [4] [5]
Appendix 6: St. Michael’s Hospital Research Ethics Board Approval

Research Ethics Office
Telephone: (416) 864-0060 Ext. 2557
Facsimile: (416) 864-0043
Email: ethico@smhc.on.ca

February 19, 2010

Dr. Alexandra Jenkins,
Risk Factor Modification Centre
St Michael’s Hospital

Dear Dr. Jenkins,

Re: REB# 09-272 - Efficacy and safety of whole grain salba (Salvia Hispanica L) on weight loss in overweight and obese individuals with Type 2 Diabetes

REB APPROVAL:

<table>
<thead>
<tr>
<th>Original Approval Date</th>
<th>February 19, 2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual/Interval Review Date</td>
<td>February 19, 2011</td>
</tr>
</tbody>
</table>

Thank you for your application submitted on October 02, 2009. At the St Michael’s Hospital (SMH) Research Ethics Board (REB) meeting held on November 04, 2009, the above referenced study was discussed and subsequently the views derived from this discussion have been documented and resolved.

The REB approves the study as it is found to comply with relevant research ethics guidelines, as well as the Ontario Personal Health Information Protection Act (PHIPA), 2004. The REB hereby issues approval for the above named study for a period of 12 months from the date of this letter. Continuation beyond that date will require further review of REB approval. In addition, the following are appropriate and hereby approved:

2. Consent Form version 3 dated January 25, 2010
3. Telephone Screening Questionnaire
4. Medical Information Form
5. Nutrition and Lifestyle Questionnaire
6. Habitual Physical Activity Questionnaire
7. Clinical Assessment Form
8. Symptoms Diary version 1 dated August 26, 2009
9. Three-Day Food Record and Hunger Scores
10. Study Poster

Further, the following documents have been received and are acknowledged:

1. Letter from Co-Investigator for site in Zagreb, Croatia
2. Canadian Diabetes Association Grant-In-Aid Application 2009
3. Beck Depression Inventory (BDI)

During the course of this investigation, any significant deviations from the approved protocol and/or unanticipated developments or significant adverse events should immediately be brought to the attention of the REB.

Dr. Alexandra Jenkins (REB# 09-272)
This letter serves as approval by the SMH REB for conduct of this study; however, additional approvals are required as outlined on the Office of Research Administration Authorization Check List form. Enclosed is a copy of this check list and REB authorization is in the appropriate space. Also, the Clinical Trial Agreements have to be submitted to the Office of Research Administration for review and approval. The remainder of the approvals must be coordinated through the Office of Research Administration prior to initiation of this research. All drug dispensing must be coordinated through the Research Pharmacy at 416-864-5413.

The St. Michael's Hospital (SMH) Research Ethics Board (REB) operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans, the Ontario Personal Health Information Protection Act, 2004, and ICH Good Clinical Practice Consolidated Guideline E6, Health Canada Part C Division 5 of the Food and Drug Regulations, Part 4 of the Natural Health Product Regulations, and the Medical Devices regulations. Furthermore, all investigational drug trials at SMH are conducted by Qualified Investigators (as defined in the letter document).

With best wishes

☐ Dr. Julie Spence
Chair, Research Ethics Board

☐ Dr. Brenda McDowell
Vice Chair, Research Ethics Board
ST MICHAEL'S HOSPITAL HEALTH SCIENCES RESEARCH PROGRAM
OFFICE OF RESEARCH ADMINISTRATION
Authorization Check List for Submission of Research Proposals and Grant Requests

Applicant(s): Dr. Alexandra Jenkins
Department: ________________________________
Funding Agency: __________ Is this proposal: New ______ Renewal ______
Type of Grant: Operating ______, Equipment ______, Personnel ______, Other ______
Full Title of Study: REBi-00-272 - Efficacy and safety of whole grain salba (Salvia Hispanica L.) on weight loss in overweight and obese individuals with Type 2 Diabetes

<table>
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<th>Proposal:</th>
<th>Yes</th>
<th>No</th>
<th>If Yes, Reviewed by:</th>
<th>Pending Approval</th>
<th>Approved</th>
<th>Authorized By</th>
</tr>
</thead>
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<td>Human Subjects to be used</td>
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<td>Biohazard Risk</td>
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<td>Animal Subjects to be used</td>
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<td>Research Vivarium Fee</td>
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<td>Does the Budget include:</td>
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<td>If Yes, Human Resource Review</td>
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<tr>
<td>Is space available to do this Research?</td>
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<td>If No, Space Allocation Committee Review</td>
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<tr>
<td>Will the Proposed Research involve the following:</td>
<td>Yes</td>
<td>No</td>
<td>If Yes, Dept Head Review:</td>
<td>Yes</td>
<td>No</td>
<td>If Yes, Dept Head Review:</td>
</tr>
<tr>
<td>Nursing Services</td>
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<td>Hematology Dept</td>
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<td>Biochemistry Dept</td>
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<td>Anaesthesia Dept</td>
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<td>Equipment Purchases</td>
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<td>If Yes, attach Quotations</td>
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<td>Equipment Maintenance</td>
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<td>If Yes, attach Quotations</td>
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SMH RESEARCH PROGRAM ADMINISTRATION:
HOSPITAL OVERHEAD CHARGES: YES ______ NO ______
MANAGER, OFFICE OF RESEARCH ADMINISTRATION
Please submit all contractual agreements for institutional approval.
DATE OF AUTHORIZATION: ________________________________

FINANCE ACCOUNTS WILL NOT BE AUTHORIZED FOR RESEARCH PROPOSALS AND GRANT REQUESTS WITHOUT PRIOR COMPLETION OF THIS FORM.
THIS APPROVAL WILL BE VALID FOR A PERIOD OF 12 MONTHS FROM THE DATE OF AUTHORIZATION.
Appendix 7: Supplement Recipe Book/Instruction Manual

Incorporating
WHOLE GRAIN
SPRINKLES
Into Your Diet

Prepared by:
The Risk Factor Modification Centre,
St. Michael’s Hospital, Toronto, ON
Appendix 8: CDA’s Beyond the Basics: Meal Planning for Healthy Eating, Diabetes Prevention and Management

### Beyond the Basics:
Meal Planning for Healthy Eating,
Diabetes Prevention and Management

#### Meal Plan

<table>
<thead>
<tr>
<th>TIME</th>
<th>CARBOHYDRATES (grams / choices)</th>
<th>GRAINS &amp; STARCHES</th>
<th>FRUITS</th>
<th>MILK &amp; ALTERNATIVES</th>
<th>OTHER CHOICES</th>
<th>VEGETABLES</th>
<th>MEAT &amp; ALTERNATIVES</th>
<th>FATS</th>
</tr>
</thead>
</table>
Appendix 9: Three-Day Food Record

3-DAY FOOD RECORD

Please record all foods and beverages as soon as possible after they are consumed so that you do not forget what you ate or drank. Record for one weekend day and two week days.

1. RECORD a description of the food or beverage using:
   - **Brand Names**
     Examples: Kellogg’s, Post, General Mills, Nabisco, Nestle, President’s Choice, Lean Cuisine, TGTBT, Campbell’s, Lipton, Becel
   - **Restaurant Names**
     Examples: McDonald’s, Swiss Chalet, Young Thailand Restaurant
   - **Cooking Method**
     Examples: raw, steamed, baked, boiled, grilled, deep-fried, pan-fried
   - **Food Form**
     Examples: fresh, canned, dried, diced, processed, skinned
   - **Food Qualities**
     Examples: low-fat, 1% milk, 2% milk, light, fat-free

2. RECORD the quantity of food or beverage consumed using:
   - **Weights** (eg: ounces, grams, litres) for all foods
     —OTHERWISE—
   - **Slices** for bread (thick or thin)
   - **Cups** for beverages, pasta, cereal, rice, mashed potatoes
   - **Small, Medium, Large** for raw fruits and vegetables
   - **Tbsp, tsp** for margarine, butter, sugar
   - **Creamers** for cream and milk
   - **Packets** for sugar
   - **Dimensions** (eg: 5cm x 5cm x 2cm) for pizza, cheese, pie, cake, meat
     (including fish and poultry)

3. RECORD descriptions and quantities of individual ingredients in mixed dishes:
   Example:

<table>
<thead>
<tr>
<th>Time</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00pm</td>
<td>Cheese Sandwich:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sesame seed bagel</td>
<td>1 medium</td>
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<tr>
<td></td>
<td>Margarine, Becel</td>
<td>1 tbsp</td>
</tr>
<tr>
<td></td>
<td>Cheese, cheddar</td>
<td>3 slices, each</td>
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<td>10cm x 10cm x 1cm</td>
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</tbody>
</table>
4. Before your largest meal of each day please complete the **Pre-Meal Hunger Score Questionnaire** and approximately 2 hours after consumption of this meal please complete the **Post-Meal Hunger Score Questionnaire**.

If you have **any** questions, please do not hesitate to contact us.
Clinical Nutrition and Risk Factor Modification Centre
70 Richmond Street East
Toronto, ON M5C 1N8
(416) 864-6060 ext 3364
### FOOD RECORD: DAY 1

<table>
<thead>
<tr>
<th>Time Eaten</th>
<th>Food/Beverage and Description (one item per line)</th>
<th>Quantity</th>
<th>CLINIC USE ONLY</th>
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<tbody>
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</tbody>
</table>

Is this a usual day? (Check the answer that applies)

- [ ] Yes
- [ ] No; please explain why: _______________________________________________________

_____________________________________________________________

_____________________________________________________________
Appendix 10: Clinical Assessment Form

**CLINICAL ASSESSMENT**

Date: ________________________________

Treatment Code (circle one): 546 824

Anthropometry and BP

<table>
<thead>
<tr>
<th>Time</th>
<th>Food item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

BP (mmHg):__________________________

Waist:Hip (cm:cm):_______:_______

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

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

Subject #: ___________

Initials: ___________
Appendix 11: Symptoms Diary

SYMPTOMS DIARY

Date: _____________________________

Please indicate in the space provided if you experience any adverse symptoms including, but not limited to, the following:

* Bloating, Belching, Diarrhea, Flatulence, Constipation, Excessive Urination, Nausea, Headache, Dizziness, Disorientation, Anxiety, Poor Wound Healing, Excessive Bleeding After Cuts, Abdominal Cramps, General Weakness*

Please rate the severity of this symptom and provide any relevant comments in the appropriate space.

<table>
<thead>
<tr>
<th>DATE</th>
<th>SYMPTOM</th>
<th>SEVERITY</th>
<th>COMMENT</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>Low 1----2-----3-----4-----5-----6-----7</td>
<td>High</td>
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<tr>
<td></td>
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<td>Low 1----2-----3-----4-----5-----6-----7</td>
<td>High</td>
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<td>Low 1----2-----3-----4-----5-----6-----7</td>
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<td>Low 1----2-----3-----4-----5-----6-----7</td>
<td>High</td>
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</tbody>
</table>

*Salvia hispanica*

LOSS Trial
Subject #: __________
Initials: __________