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

by

Christy Elizabeth Brissette

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

Department of Nutritional Sciences

University of Toronto

© Copyright by Christy Brissette 2013
The Effect of *Salvia hispanica* L. Seeds on Weight Loss in Overweight and Obese Individuals with Type 2 Diabetes Mellitus

Christy Brissette

Master of Science, 2013

Department of Nutritional Sciences

University of Toronto

Abstract

There is growing interest in the potential role of omega-3/fibre-rich seeds in attenuating obesity and other cardiovascular disease (CVD) risk factors in individuals with type 2 diabetes mellitus (T2DM). Preliminary data suggests that consumption of white *Salvia hispanica* L. (Salba®) seeds prolongs satiety and may aid weight loss. This randomized, double-blind, parallel study assessed the efficacy and safety of *Salvia hispanica* in overweight/obese individuals with T2DM on weight, body composition, glycemic control and other CVD risk factors. Fifty-eight participants consumed a hypocaloric diet including *Salvia hispanica* or an energy-and-fibre-matched control over 24 weeks. Greater reductions in weight, waist circumference and inflammation occurred in the *Salvia hispanica* group versus control. There were no significant between-group differences in safety parameters, glycemic control or other CVD risk factors. *Salvia hispanica* seeds may support weight loss in overweight/obese individuals with T2DM. Further research is needed to determine whether these effects are maintained.
Acknowledgments

Thank you to my supervisors, Dr. Vladmir Vuksan and Dr. Alexandra Jenkins, for their support and guidance throughout the research process and during my career development. I would also like to thank my advisory committee members, Dr. Thomas Wolever and Dr. Pauline Darling, for their valuable feedback and insightful comments.

My family and friends deserve accolades for their ongoing patience, support and understanding over the past two years. I would also like to thank my Naim for keeping my motivation and spirits high. My mom deserves special recognition for providing the encouragement and humour that often kept me going. Thank you to my dad for teaching me the value of hard work and a curious mind. I would like to think he would read this thesis cover to cover. My sincere thanks also goes to my colleagues and the volunteers and co-op students at the Risk Factor Modification Centre. They were a pleasure to work with and provided invaluable support along the way. In particular, Allison Komishon, Sam Cooper, Jennifer Chang and Lauryn Choleva deserve recognition for their friendship and contributions to this work.

Last but certainly not least, the study participants volunteered their time and without them, this study would not have been possible. I am inspired by their desire to take charge of their health by taking this opportunity to learn more about the impact of nutrition.
# Table of Contents

Abstract .......................................................................................................................... ii

Acknowledgements ........................................................................................................ iii

Table of Contents .......................................................................................................... iv

List of Tables .................................................................................................................. ix

List of Figures ................................................................................................................. xi

List of Appendices ......................................................................................................... xii

List of Abbreviations ................................................................................................... xiii

Chapter 1: Introduction ...................................................................................................... 1

1.1 Introduction ............................................................................................................... 1

Chapter 2: Literature Review .............................................................................................. 3

2.1 Obesity ..................................................................................................................... 3

2.1.1 Complications of Obesity ..................................................................................... 6

2.1.1.1 Cardiovascular Disease ..................................................................................... 7

2.1.1.2 Type 2 Diabetes Mellitus .................................................................................. 9

2.1.1.2.1 Diagnosis and Management of T2DM ............................................................ 10

2.1.1.2.2 Obesity and T2DM ....................................................................................... 12

2.1.1.2.3 Inflammation ............................................................................................... 13

2.1.2 Obesity Management .......................................................................................... 14

2.1.2.1 Pharmacological Approaches to Weight Loss .................................................... 15
4.4 Inclusion and Exclusion Criteria.................................................................41

4.5 Study Intervention.....................................................................................42

4.6 Study Protocol and Timeline....................................................................46

4.7 Study Measurements..................................................................................48

4.7.1 Anthropometric Assessment.................................................................48

4.7.2 Office Blood Pressure............................................................................50

4.7.3 Blood Samples.........................................................................................50

4.7.3.1 Glycemic Parameters.........................................................................50

4.7.3.2 Lipid Parameters..................................................................................51

4.7.3.3 Inflammation.......................................................................................52

4.7.3.4 Satiety-Related Hormones .................................................................52

4.7.4 Safety Parameters...................................................................................53

4.7.5 Compliance.............................................................................................55

4.7.5.1 Supplement Consumption.................................................................55

4.7.5.2 Diet Analysis........................................................................................56

4.5 Statistical Analysis....................................................................................56

Chapter 5: Results..........................................................................................58

5.1 Study Participants.......................................................................................58

5.2 Treatment of Missing Data.......................................................................62

5.3 Efficacy of Salba........................................................................................63

5.3.1 Effect on Primary Outcome Measure....................................................63
List of Tables

2-1. The nutritional composition of *Salvia hispanica* (Salba)……………………………………….22

2-2. The amino acid composition of Salba………………………………………………………29

4-1. The nutritional composition of the study supplements……………………………………..45

4-2. The nutritional composition of the study bread ..........................................................45

4-3. Protocol of measurements conducted at each visit....................................................49

5-1. Baseline subject characteristics..................................................................................61

5-2. The effect of Salba, compared to oat bran-based control, on body weight, waist and hip circumference and % body fat measured by bioelectrical impedance at middle and end of treatment compared to baseline ..........................................................................................................................64

5-3. The effect of Salba, compared to oat bran-based control, on fasting blood glucose, glycated hemoglobin and fasting insulin at middle and end of treatment compared to baseline ..........................................................................................................................67

5-4. The effect of Salba, compared to oat bran-based control, on total body fat percentage and regional fat distribution as measured by dual energy x-ray absorptiometry at middle and end of treatment compared to baseline ..........................................................................................................................72

5-5. The effect of Salba, compared to oat bran-based control, on office blood pressure at middle and end of treatment compared to baseline .......................................................78

5-6. The effect of Salba, compared to oat bran-based control, on fasting serum lipids and inflammation at middle and end of treatment compared to baseline ..........................................................................................................................79

5-7. The effect of Salba, compared to oat bran-based control, on fasting ghrelin, adiponectin and PYY levels at middle and end of treatment compared to baseline .........................82

5-8. The effect of Salba, compared to oat bran-based control, on kidney and liver function and bleeding time at middle and end of treatment compared to baseline .................85
5-9. The number of participants who reported symptoms at baseline, middle and end of the study in each of the treatment groups

5-10. Comparison of supplement consumption as reported by study participants to prescribed supplement amount during the first half of the study period, the second half of the study period and overall in the Salba and oat bran groups

5-11. Comparison of plasma phospholipid fatty acid content between treatment groups at the end of the study

5-12. Comparison of the nutritional composition of the diet including study supplements between each of the treatment groups at baseline, middle and end of treatment as reported by participants in 3-day food records
List of Figures

4-1. Study timeline ........................................................................................................................................47

5-1. Recruitment flowchart from initial contact until study completion with inclusion of participants who completed week 18 .............................................................................................................60

5-2. The effect of Salba, compared to oat bran-based control, on change in body weight at middle and end of treatment compared to baseline .............................................................................................................65

5-3. The effect of Salba, compared to oat bran, on A1C at middle and end of treatment, compared to baseline .............................................................................................................................................68

5-4. The effect of Salba, compared to oat bran, on fasting serum glucose levels at baseline, middle, and end of treatment .............................................................................................................................................69

5-5. The effect of Salba, compared to oat bran, on fasting serum insulin levels at baseline, middle, and end of treatment .............................................................................................................................................70

5-6. The effect of Salba, compared to oat bran, on change in %BF by region as measured by DXA, compared to baseline .............................................................................................................................................73

5-7. The effect of Salba compared to oat bran on change from baseline in total body weight, lean mass and fat mass in kilograms as measured by DXA .............................................................................................................................................74

5-8. The effect of Salba, compared to oat bran, on change in waist circumference from baseline .............................................................................................................................................75

5-9. The effect of Salba, compared to oat bran, on change in fasting CRP levels from baseline .............................................................................................................................................79

5-10. The effect of Salba, compared to oat bran, on changes in fasting ghrelin, adiponectin and PYY levels from baseline .............................................................................................................................................82
List of Appendices

**Appendix 1** Telephone Screening Questionnaire ......................................................... 134

**Appendix 2** Informed Consent Form ............................................................................. 136

**Appendix 3** Medical Information Form ......................................................................... 144

**Appendix 4** Dietary Questionnaire .................................................................................. 151

**Appendix 5** Physical Activity Questionnaire ................................................................. 155

**Appendix 6** St. Michael’s Hospital Research Ethics Board Original Approval .............. 157

**Appendix 7** Recipe Book/Instruction Manual ................................................................. 158

**Appendix 8** CDA’s Beyond the Basics: Meal Planning for Healthy Eating, Diabetes Prevention and Management ................................. 159

**Appendix 9** Three Day Food Record .............................................................................. 160

**Appendix 10** Clinical Assessment Form ......................................................................... 162

**Appendix 11** Symptoms Diary ...................................................................................... 163
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%BF</td>
<td>Percent Body Fat</td>
</tr>
<tr>
<td>A1C</td>
<td>Glycated Hemoglobin</td>
</tr>
<tr>
<td>A:G</td>
<td>Ratio of % Android body fat to % Gynoid body fat</td>
</tr>
<tr>
<td>AEBSF</td>
<td>4-(2-Aminoethyl) Benzenesulfonfyl Fluoride</td>
</tr>
<tr>
<td>AHA</td>
<td>Anti-Hyperglycemic Agent</td>
</tr>
<tr>
<td>AHEAD</td>
<td>Action for Health in Diabetes study</td>
</tr>
<tr>
<td>AHTN</td>
<td>Anti-hypertensive Medication</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>ARA</td>
<td>Arachidonic Acid</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>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>CCHS</td>
<td>Canadian Community Health Survey</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>CHARGE</td>
<td>Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
</tr>
<tr>
<td>CHMS</td>
<td>Canadian Health Measures Survey</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatinine</td>
</tr>
<tr>
<td>CRP</td>
<td>High-Sensitivity C-Reactive Protein</td>
</tr>
<tr>
<td>CV</td>
<td>Cardiovascular</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic Blood Pressure</td>
</tr>
<tr>
<td>DCCT</td>
<td>Diabetes Control and Complications Trial</td>
</tr>
<tr>
<td>DRIs</td>
<td>Dietary Reference Intakes</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual Energy X-Ray Absorptiometry</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaneoic Acid</td>
</tr>
<tr>
<td>EER</td>
<td>Estimated Energy Requirements</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EPA</td>
<td>Eicosapentaneoic Acid</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization</td>
</tr>
<tr>
<td>FBG</td>
<td>Fasting Blood Glucose</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FFA</td>
<td>Free Fatty Acid</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Glucagon-Like Peptide-1</td>
</tr>
<tr>
<td>GI</td>
<td>Glycemic Index</td>
</tr>
<tr>
<td>HDL</td>
<td>High-Density Lipoprotein</td>
</tr>
<tr>
<td>HDL-C</td>
<td>Total Cholesterol to HDL Ratio</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>iAUC</td>
<td>Incremental Area Under the Curve</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalized Ratio</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin Resistance</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic Acid</td>
</tr>
<tr>
<td>LDL</td>
<td>Low-Density Lipoprotein</td>
</tr>
<tr>
<td>LLA</td>
<td>Lipid Lowering Agent</td>
</tr>
<tr>
<td>MI</td>
<td>Myocardial Infarction</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated Fatty Acids</td>
</tr>
<tr>
<td>n-3</td>
<td>Omega 3 Polyunsaturated Fatty Acid</td>
</tr>
<tr>
<td>n-6</td>
<td>Omega 6 Polyunsaturated Fatty Acid</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide Adenine Dinucleotide</td>
</tr>
<tr>
<td>NCSS</td>
<td>Number Cruncher Statistical System</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NS</td>
<td>Not Statistically Significant</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>PDCAAS</td>
<td>Protein Digestibility Corrected Amino Acid Score</td>
</tr>
<tr>
<td>PG</td>
<td>Plasma Glucose</td>
</tr>
<tr>
<td>PPG</td>
<td>Postprandial Glycemia</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin Time</td>
</tr>
<tr>
<td>PUFAs</td>
<td>Polyunsaturated Fatty Acids</td>
</tr>
<tr>
<td>PYY</td>
<td>Peptide Tyrosine Tyrosine</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomized Controlled Trial</td>
</tr>
<tr>
<td>RD</td>
<td>Registered Dietitian</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>SE</td>
<td>Standard Error of the Mean</td>
</tr>
<tr>
<td>SFA</td>
<td>Saturated Fatty Acids</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>TAGs</td>
<td>Triglycerides</td>
</tr>
<tr>
<td>TC</td>
<td>Total Cholesterol</td>
</tr>
<tr>
<td>TEF</td>
<td>Thermic Effect of Food</td>
</tr>
<tr>
<td>TSQ</td>
<td>Telephone Screening Questionnaire</td>
</tr>
<tr>
<td>UKPDS</td>
<td>United Kingdom Prospective Diabetes Study</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
</tr>
<tr>
<td>vWF</td>
<td>Von Willebrand Factor</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>W:H</td>
<td>Waist-to-Hip ratio</td>
</tr>
</tbody>
</table>
Chapter 1
Introduction

1.1 Introduction

The prevalence of obesity in Canada and worldwide continues to rise despite international efforts to intervene. While excess body weight is essentially caused by energy intake exceeding energy expenditure, the etiology of obesity appears to be a conglomeration of many behavioural and physiological factors. Possible factors driving the obesity pandemic include clusters of societal changes promoting greater intake of energy-dense, processed foods and increasingly sedentary lifestyles. Rising obesity rates are of particular concern as obesity contributes to the development of chronic diseases such as type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and several types of cancer. Obesity and its complications are one of the leading causes for physician visits in Ontario, resulting in direct and indirect costs to the Canadian healthcare system of nearly 5 billion dollars per annum and rising (1).

While pharmacological and surgical treatments for obesity are available, they may cause serious side effects and are often only recommended in extreme cases. Lifestyle interventions, particularly dietary changes, are recommended as first line treatment for overweight and obesity (2). The simple expedient of cutting energy intake by reducing food intake leads to increased feelings of hunger and a subsequent return to the customary dietary intake and rebound weight gain. As such, foods that may promote satiety and improve the efficacy and acceptability of hypocaloric diets are an area of interest. One food that has shown potential as a weight management aid is *Salvia hispanica* L., an oily seed commonly known as chia.

*Salvia hispanica* seeds are high in protein, dietary fibre and omega-3 fatty acids, contain little available carbohydrate, and are rich in minerals such as calcium. Individually, these nutrients
have been shown to promote weight control and in some cases, improve CVD risk factors, suggesting the potential usefulness of including *Salvia hispanica* in the diets of individuals with excess body weight, T2DM, or both. Preliminary data suggests *Salvia hispanica* may help promote satiety and improve CVD risk factors such as CRP, a marker of inflammation. Thus, *Salvia hispanica* may be helpful in supporting weight loss and reducing CVD risk in individuals with T2DM when part of an energy-reduced diet.

The current study investigated whether a single, standardized variety of *Salvia hispanica*, commercially known as Salba®, is safe and effective for promoting weight loss as part of an energy-reduced diet in overweight and obese individuals with T2DM. The impact of *Salvia hispanica* on safety parameters, glycemic control and other CVD risk factors compared to control was also assessed. If the inclusion of *Salvia hispanica* in a hypocaloric diet proves to be effective and safe in supporting weight loss while improving glycemic control and CVD risk factors in overweight and obese individuals with T2DM, these seeds may be a useful supplement to conventional treatment in this population.
Chapter 2
Literature Review

2.1 Obesity

Obesity has become a global epidemic, with the World Health Organization (WHO) estimating that at least 1 billion adults worldwide are overweight and more than 300 million are obese (3). Body Mass Index (BMI) is a calculation used to estimate weight-associated health risks in populations between the ages of 18 and 65, and is calculated by dividing a person’s weight in kilograms by his or her height in meters squared. Overweight is defined as having a BMI of 25.0 - 29.9 kg/m$^2$ inclusive, while obesity is defined as a BMI of 30 kg/m$^2$ or greater. Obesity is further subdivided into classes, with escalating risk of chronic disease the higher the obesity class. The obesity classes are defined by the following cut-offs: Class I: 30.0-34.9 kg/m$^2$; Class II: 35.0-39.9 kg/m$^2$; and Class III: ≥40.0 kg/m$^2$ (4). According to measured heights and weights collected from the Canadian Community Health Survey (CCHS) and the Canadian Health Measures Survey (CHMS), 62.1% of Canadian adults were overweight, with nearly half of these adults classified as clinically obese as of 2008 (5-7).

In individuals, it is recommended that BMI be used in combination with waist circumference in order to account for musculature and fat mass to more accurately assess chronic disease risk (8). Abdominal adiposity is independently associated with a higher risk of developing type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (9). According to the WHO and National Institutes of Health (10), a waist circumference ≥102 cm (40 inches) in men and ≥88 cm (35 inches) in women is associated with increased chronic disease risk (3,11). Because these cut-offs were determined using primarily Caucasian populations, and differences in frame size and fat distribution patterns may impact health risks, additional cut-offs based on ethnicity have also been suggested (12). The Heart and Stroke Foundation of Canada recommends that 90 cm (35
inches) for men and 80 cm (32 inches) for women be used as waist circumference cut-offs in South Asian and Asian populations (13).

Based on the 2007-2009 CHMS, the prevalence of waist circumference measurements above the cut-off in ages 20-39 years, 40-59 years and 60-69 years was 21%, 38% and 52% for men and 31%, 47% and 65% for women (5). These findings indicate that waist circumference may increase with age, and that the majority of Canadians above the age of 60 are at high risk of health problems attributable to abdominal adiposity.

At first glance, the etiology of obesity appears to be quite simple. Weight gain is essentially caused by positive energy balance, or energy intake exceeding energy expenditure. However, the underlying cause of the increasing disparity in energy balance is difficult to ascertain. While genetics likely play a role, the development of obesity seems to be attributable to a complex interplay between environmental and societal factors such as the rise in availability of energy-dense foods and increasingly sedentary lifestyles (14). The complexity of the problem renders it a challenging one for public health agencies and healthcare providers to address (15).

Evidence linking worsening diet quality with the rise in obesity is strong, with studies of Canadian energy intake patterns from 1976 – 2003 showing significant increases in energy consumption, mainly attributable to soft drinks, refined carbohydrates and saturated fat in processed foods and animal products (16). Several studies have used low fruit and vegetable intake as an indicator of poor diet quality, finding a strong association with obesity (17,18).

A growing area of research into the etiology of obesity is examining the role of satiety-regulating hormones such as ghrelin, adiponectin and PYY in affecting energy intake in lean and obese individuals. Ghrelin increases food intake, while adiponectin and PYY promote satiety. Serum ghrelin levels rise prior to eating and decline post-prandially (19). In obese individuals, fasting
levels of ghrelin are significantly reduced compared to lean individuals (20). Fasting ghrelin is also reduced in T2DM compared to individuals without the disease (21). In individuals with T2DM, ghrelin levels have been shown to be negatively correlated with BMI, visceral fat and fasting insulin levels (22). Weight loss increases ghrelin levels, with greater amounts of weight loss being positively correlated with the extent of the increase in ghrelin levels (23,24). Unlike some of the other satiety-regulating hormones, the current literature negates the presence of sex differences in ghrelin levels (25,26).

Adiponectin is a protein synthesized by white adipocytes and exhibits insulin-sensitizing, anti-inflammatory and antiatherogenic effects (27). Levels of adiponectin are lower in women than in men, although research by Putz et al. suggests that sex differences in adiponectin levels may not be present in T2DM (28). Adiponectin levels have been shown to be lower in individuals with T2DM and are further reduced in those with both T2DM and coronary artery disease (CAD). In a multivariate analysis, fasting insulin levels did not exhibit an independent effect on adiponectin levels, while BMI, triglyceride levels and CAD were shown to be significantly related to adiponectin concentrations (28,29). Weight loss has been shown to increase adiponectin levels in individuals with T2DM and in healthy participants (30).

PYY is a peptide consisting of 36 amino acids (31). It is most abundant in the colon and rectum, although it is secreted along the entire gastrointestinal tract (32). Serum PYY is lowest in the fasted state and increases post-prandially, peaking around 1-2 hours after a meal. Fasting PYY levels are lower in obese individuals compared to lean controls (33). In both lean and obese individuals, administration of exogenous PYY has been shown to promote satiety and reduce energy intake (34,35). Further, fasting PYY levels have been shown to negatively correlate with BMI and waist circumference (36). Weight loss has been shown to increase fasting PYY levels which may help to promote maintenance of weight loss (37). While the impact of satiety-
regulating hormones on efficacy of weight loss strategies in T2DM is complex, trends in levels of these hormones over time may offer insights into the mechanistic effects of fibre supplementation as part of an energy-reduced diet.

The inverse relationship between leisure time physical activity and obesity has been well documented (18). Canadian guidelines recommend that adults ages 18-64 engage in moderate to vigorous aerobic physical activity for at least 150 minutes per week to maintain a healthy body weight and reduce chronic disease risk (38). Self-report data from the 2007/2008 CCHS suggests that only half (51%) of Canadians ages 12 and older were at least moderately active (7). However, data collected from the 2007-2009 CHMS suggest that only 15% of Canadians are actually meeting the recommendations for physical activity (10).

Without lifestyle intervention, obese individuals will continue to gain weight over time (39). A longitudinal cohort study followed 3 325 African American and Caucasian men and women between the ages of 18 – 30 over 10 years. The authors found that the mean±SD weight gain in African American men and women was 10.5±10.0 kg and 11.7±11.0 kg, while in Caucasian men and women, weight gain was 7.7±8.0 kg and 7.2±10.0 kg, respectively. Weight gain was associated with adverse changes in low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TAGs), fasting insulin and blood pressure (BP) among all groups, even in those who were at a healthy body weight (39). This research underscores the need for interventions in overweight and obese individuals to prevent further increases in weight that may negatively impact CVD risk.

### 2.1.1 Complications of Obesity

Obesity is associated with increased morbidity and mortality (14,40). A recent systematic review found associations between obesity and the incidence of chronic diseases and conditions such as
T2DM, CVD (including hypertension, CAD, congestive heart failure and stroke), gallbladder
disease, asthma, chronic back pain, osteoarthritis and cancers of the pancreas, breast,
endometrium, ovaries, colon and kidneys (41). Psychological conditions such as depression,
reduced self-esteem and poor quality of life are also more common in obese individuals (2,42).
An analysis of data from the National Health and Nutrition Examination Survey (43) I and II in
the U.S. found a positive relationship between fat mass and mortality from all causes in men,
whereas fat-free mass appeared to be protective (43).

A study combining data from the 1994/95 and 1996/97 National Population Health Surveys, the
Public Health Agency of Canada’s Economic Burden of Illness Database created in 2000, and
CCHS from 2000-2008 evaluated both direct and indirect costs to the health care system from
obesity. The report concluded that from 2000 to 2008, the economic burden of obesity in Canada
increased by $735 million, from $3.9 to $4.6 billion per year (44). A similar study estimated the
annual economic impact in Canada of 18 chronic diseases strongly associated with obesity to be
up to $7.1 billion in 2006 dollars (1).

2.1.1.1 Cardiovascular Disease

Weight loss has been shown to play an important role in CVD risk reduction by reducing blood
pressure, improving lipid levels and reducing markers of inflammation such as high-sensitivity C-
reactive protein (CRP).

Hypertension prevention and treatment are vital to reduce the risk of CVD morbidity and
mortality (45). Blood pressure often rises with increasing body weight, and lifestyle interventions
resulting in weight loss have been shown to lead to improvements in hypertension (46,47).
Results of the Nurses’ Health Study conducted in 80,000 women found that gaining 5 kg was
associated with a 60% higher relative risk of developing hypertension compared to women who
gained 2 kg or less (48). Analysis of data from the Framingham Cohort estimated that obesity may be an independent predictor of hypertension in 78% of men and 65% of women (49). A recent systematic review of the impact of weight loss via lifestyle interventions on blood pressure identified 8 clinical trials and 8 cohort studies with a follow up of 2 years or more. Overall, the impact of weight loss on changes on diastolic blood pressure (DBP) was not statistically significant. For systolic blood pressure (SBP), in studies lasting 2-3 years, SBP was reduced by 1 mm Hg for every 1 kg lost (50). These findings support the findings of a previous meta-analysis of 25 studies on the subject (46). As such, dietary interventions aiding weight loss are also likely to reduce SBP and subsequently lower CVD risk.

Weight loss of 5-10% of body weight is recommended by the NIH based on associated reductions in CVD risk factors in otherwise healthy populations (51,52), but few studies have identified whether this holds true in individuals with T2DM (53). Analysis of data from the Look AHEAD (Action for Health in Diabetes) study conducted in 5,145 men and women of diverse ethnic backgrounds with T2DM revealed that compared to weight-stable subjects, individuals who lost 5-10% of their body weight over 1 year had a greater chance of reducing A1C by 0.5%, reducing SBP by 5 mm Hg, DBP by 5 mm Hg, raising HDL by 0.1293 mmol/L and reducing TAGs by 0.4516 mmol/L (53). These results indicate that modest weight loss may benefit individuals with T2DM by improving markers of CVD risk.

Obesity places additional stress on the CV system, as excess body weight increases total blood volume and cardiac output, increasing cardiac workload (54). Strong evidence substantiates obesity as an independent risk factor for CAD and CVD events (55,56). Results from the landmark Framingham Heart Study demonstrated that in obese individuals, the risk of heart failure is doubled compared to lean individuals (57). This may be partially attributable to the endocrine functions of adipocytes. Adipocytes produce leptin, a hormone that influences energy
intake and metabolism and may impact CVD risk (58). Furthermore, the elevation of inflammatory markers such as CRP often seen in obesity may lead to leptin resistance, resulting in the loss of effect of elevated leptin levels in reducing energy intake or increasing energy expenditure (59).

In addition to hypertension and T2DM, dyslipidemia plays a role in increasing CAD risk (60,61). Dyslipidemia is defined as elevated levels of plasma triglycerides (TAGs) and low-density lipoprotein (LDL) accompanied by low levels of high-density lipoprotein (HDL). This lipid profile has been associated with increased risk of CAD, which has been shown to increase the risk of heart attack and stroke (62).

Diet can lead to additional immune activation against the backdrop of obesity, leading to further inflammation. For example, a high fat diet has been shown to activate inflammatory signaling pathways in the hypothalamus, leading to greater food intake and fat storage (63). CRP appears to be a helpful marker of systemic inflammation, with recent research showing that weight loss is associated with reductions in CRP (64). As such, dietary interventions that promote weight loss may help to reduce the inflammatory burden of obesity and T2DM, which may play a role in the prevention of CVD.

2.1.1.2 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus is a metabolic chronic disease whereby insulin resistance, insulin deficiency, or a combination of the two, result in elevated blood glucose levels (65). Chronic hyperglycemia is associated with greater risk of complications due to damage to the kidneys, nerves, eyes, heart and blood vessels, resulting in organ dysfunction and development of additional chronic diseases (66). Cardiovascular disease is the primary cause of death in T2DM (67-69). As such, optimal glycemic control combined with the treatment of CVD risk factors
such as obesity, hypertension and dyslipidemia are essential to reducing morbidity and mortality in individuals with T2DM (66,70,71).

2.1.1.2.1 Diagnosis and Management of T2DM

Findings from epidemiological studies show that A1C levels >7.0% are associated with a significant increased risk of microvascular and macrovascular complications (72-74). Results from the Diabetes Control and Complications Trial (DCCT) (73) and the United Kingdom Prospective Diabetes Study (UKPDS) (74) showed a positive relationship between A1C and T2DM complications. In the DCCT, reducing A1C from 8.0 to 7.2% was associated with reducing the risk of retinopathy by half (73). The results of the UKPDS showed that each 1% reduction in A1C was associated with a 37% reduction in the risk of microvascular complications, a 14% reduction in risk of myocardial infarctions (MI), and a reduction in the risk of T2DM-related and all-cause mortality (74).

Both fasting and postprandial glucose levels are associated with increased risk of T2DM-related complications (75). In a meta-regression analysis of 20 studies including nearly 100,000 participants for a mean follow-up period of 12.4 years, fasting glucose was shown to have a direct relationship to CV events. The risk of CV events appears to increase with fasting glucose levels even when they are within the normal range in individuals without T2DM (76). Based on the current evidence, the Canadian Diabetes Association (77) Clinical Practice Guidelines (78) recommend using an A1C ≤7.0% as a glycemic control target, achieved by aiming for fasting blood glucose readings between 4.0 - 7.0 mmol/L, and 2 hour postprandial glucose levels of 5.0 - 10.0 mmol/L. If A1C targets are not being met, goals for postprandial glucose levels should be lowered to 5.0 - 8.0 mmol/L (78). These goals may need to be adjusted on an individual basis based on the physician's clinical judgment, weighing both the costs and benefits of tighter glycemic control and characteristics of the individual patient, such as age and comorbidities (78).
Positive lifestyle changes to promote a healthy body weight are required on an ongoing basis to manage T2DM. In some cases, particularly in the early stages of T2DM, glycemic control may be achieved through lifestyle interventions (diet and exercise) alone (78). In 2011, Statistics Canada conducted a survey of a representative sample of nearly 3000 individuals entitled “Living with Chronic Diseases in Canada” (79). Of the respondents with T2DM, 89% reported changing their diet to improve glycemic control and 70% reported trying to be more physically active. Of the 77% of respondents with T2DM who self-identified as being overweight or obese, 80% stated they had attempted to lose weight in the past (79).

Despite these efforts, results of the Diabetes in Canada Evaluation Study, a cross-sectional study of the glycemic control of nearly 2500 Canadians with T2DM, revealed that 49% of the patients were not achieving A1C targets of <7.0% (80). These findings are supported by American NHANES data which demonstrated that despite national efforts to improve glycemic control, over 40% of Americans with T2DM remain above target (81).

At the time of diagnosis, the CDA Clinical Practice Guidelines recommend initiating lifestyle interventions with or without metformin. For individuals with A1C values ≥8.5%, it is recommended that metformin be started immediately and that the clinician also consider adding another oral anti-hyperglycemic medication. For individuals with A1C values <8.5%, lifestyle changes are encouraged with or without initiation of metformin. If glycemic control is not achieved within 2 to 3 months of lifestyle intervention, the addition of oral agents or an increase in the dosage of metformin is recommended (78). Data from the CCHS collected in 2009-2010 revealed that 85.1% of individuals 20 years or older with T2DM reported being on at least one oral medication, insulin, or both (79). Oral agents for the treatment of T2DM offer various modes of action, including increasing insulin secretion or insulin sensitivity, decreasing the rate of glucose absorption, or suppressing hepatic glucose production (82). Metformin is recommended
as first-line pharmacological treatment for most patients, as it has been shown to effectively reduce A1C and is weight-neutral or may promote weight loss in overweight individuals (83). This is particularly important as many of the pharmacological anti-hyperglycemic therapies can lead to weight gain, further exacerbating IR (65).

2.1.1.2.2 Obesity and T2DM

Obesity is a key risk factor for developing T2DM. In the Nurses’ Health Study, the risk for developing T2DM was assessed prospectively by correlating BMI at age 18, BMI at ages 30 – 55 and diagnosis of T2DM. The risk of developing T2DM increased from a standardized 1.0 for a BMI <22 kg/m² at age 18 to a relative risk of 1.93 for those with a BMI ≥35 by age 30 – 55 years (84). It is therefore not surprising that over 80% of individuals with T2DM are overweight or obese (53).

Weight loss has been shown to improve blood glucose control (53,85) by increasing insulin sensitivity and cellular glucose uptake, while also reducing the release of glucose by the liver (86,87). Initiation of an energy-reduced diet appears to immediately improve insulin sensitivity, even before weight loss occurs (88). This suggests that restriction of energy intake can help to improve hyperglycemia independent of weight loss. However, energy restriction is difficult to maintain over the long-term. In addition, weight control appears to be particularly challenging for individuals with T2DM. In a weight loss trial comparing the efficacy of two dietary interventions in overweight and obese subjects with either hypertension and dyslipidemia or T2DM, individuals with T2DM lost half as much weight as those without T2DM after 1 year, regardless of the diet type followed (89). A meta-analysis of weight loss randomized controlled trials (RCTs) revealed that in studies that included subjects with T2DM, half as much weight was lost compared to studies in participants without T2DM. Overall, subjects with T2DM lost less than half of the mean BMI units than those without the disease. Weight change in individuals
with T2DM was significantly less than in those without T2DM at every quarterly time point, from 3 months to 1 year of weight loss treatment (90).

### 2.1.1.2.3 Inflammation

While the unifying mechanisms between obesity and T2DM are not well understood, it has been noted that obesity and co-morbidities such as T2DM and CVD share a state of chronic, low-grade inflammation impacting multiple organ systems (91). This inflammatory state may be attributable to increased pro-inflammatory signaling from adipocytes that occurs with excess adiposity (92). A cross-sectional study of overweight men and women (n=923) found that higher levels of CRP, an acute phase protein and marker of inflammation, were associated with reduced insulin sensitivity and increased IR. The authors postulated that low-grade inflammation is more likely to be an outcome of obesity rather than the cause (93).

Analysis of gene expression in adipocytes reveals an overexpression of inflammatory genes associated with IR and obesity (94,95). As a result, serum levels of acute phase proteins, such as CRP, are elevated in obesity and metabolic disease states (96). Obesity appears to cause a shift from homeostatic immune signals towards pro-inflammatory signals in the brain and adipose tissue that lead to derangements in insulin sensitivity. Obese individuals have been shown to have higher concentrations of circulating free fatty acids compared to lean individuals, which appears to correspond with increases in inflammatory mediators that may lead to IR and eventually, T2DM (92,96).

Body fat percentage appears to be the primary determinant of levels of inflammatory markers such as CRP (97), with studies linking each excess kilogram of body fat with the accumulation of up to 30 million macrophages, a measure of immune system activity (98). Not surprisingly, CRP levels have been shown to be higher in obese individuals with T2DM than in lean individuals.
with T2DM (99). Over time, increases in circulating free fatty acids and cytokines, together with decreased adiponectin, may eventually lead to atherosclerosis in the blood vessels and lipid accumulation in the muscle, increasing IR. In the islet cells of the pancreas, inflammation can reduce insulin secretion and trigger β-cell apoptosis (100,101). Taken together, these findings suggest that T2DM may be an independent predictor of weight loss and maintenance success (90), further supporting the need for novel therapies that are easy to administer and will augment the effectiveness of an energy-reduced diet.

It has been demonstrated that T2DM and obesity independently increase CVD risk, and when occurring simultaneously, further compound CVD risk factors such as hypertension, dyslipidemia and inflammation (102). The 2006 Canadian Obesity Guidelines recommend achieving and maintaining a reduced body weight and preventing further weight gain as important treatment goals for overweight and obese individuals with T2DM. Weight loss has been shown to improve glycemic control (87), improve CVD risk factors (86) and reduce the risk of mortality in individuals with T2DM (47,103). These findings underscore the importance of reducing CVD risk in this vulnerable population through dietary strategies promoting weight loss.

### 2.1.2 Obesity Management

Dietary intervention, with the goal of achieving negative energy balance, remains a fundamental strategy in the management of obesity (104). Current guidelines recommend a diet planned to help achieve an energy deficit of 500 to 1,000 kcal/day with the aim of achieving a healthy rate of weight loss of 0.5 - 1.0 kg/week. Greater energy restrictions are often short-lived and generally unsuccessful in achieving weight loss over the long term (29). In individuals with T2DM, weight management is even more challenging, in part because of the weight-promoting effects of many glucose-lowering therapies (i.e. insulin, thiazolidinediones and sulfonylureas). A modest amount of steady weight loss, defined as 5-10% of initial body weight, has been shown to substantially
improve T2DM control and its associated risk factors (105-107). Current treatment options are not without their caveats: adhering to lifestyle interventions can be incredibly challenging, and the side effects and risks of pharmacological and surgical treatments must be considered along with their benefits (108-110).

### 2.1.2.1 Pharmacological Approaches to Weight Loss

Pharmacotherapy is recommended in individuals with a BMI ≥30 kg/m² or with a BMI ≥27 kg/m² and the presence of additional risk factors for T2DM (i.e. family history, impaired fasting glucose or impaired glucose tolerance) or CVD risk factors (i.e. dyslipidemia, hypertension) who have tried lifestyle changes and had limited success after 3-6 months. The 2006 Clinical Practice Guidelines for obesity management published by Obesity Canada suggest that individuals with T2DM who are overweight or obese and unable to lose sufficient weight with diet and exercise add an anti-obesity medication to support their lifestyle efforts (2).

In Canada, only one anti-obesity medication is currently approved for long-term use: orlistat (sold under the trade name Xenical®). Orlistat works by inhibiting the action of gastric and pancreatic lipases needed for digestion of dietary fats. As a result, fat absorption is decreased by ~30%, and the undigested fat passes through the intestine and is excreted. The resultant reduction in energy absorption promotes an energy deficit and subsequent weight loss (111,112).

The drawbacks of orlistat include its unpleasant side effects, the most common of which include abdominal pain, diarrhea, oily spotting, fecal urgency and incontinence and rectal bleeding (113). Further issues with orlistat include reduced absorption of fat-soluble vitamins A, D, E and K and beta-carotene, an antioxidant that acts as a precursor to vitamin A (110). Malabsorption of these nutrients further increases the risk of nutrient deficiency in a population of dieters already at high risk, as energy restriction is often paired with nutrient restriction. The drug monograph cautions
physicians and patients that it must be used as an adjunct to, and not a replacement for, a lower fat diet (<30% of daily energy intake) and that replacing fat intake with excess kilocalories from protein or carbohydrates will prevent weight loss success (113).

Other anti-obesity medications currently available or under development target the action of gut hormones (114). Among the gut hormones, the action of glucagon-like peptide-1 (GLP-1) has been the most studied in individuals with T2DM. GLP-1 is an incretin hormone released in the gastrointestinal tract by L cells in the small intestine. GLP-1 agonists are primarily used in T2DM management, as GLP-1 helps to regulate the release of insulin and glucagon (115). However, GLP-1 may also promote weight loss, as it has been shown to delay gastric emptying and impacts the satiety centre in the brain, leading to increased satiety and reduced energy intake (116). An RCT conducted in individuals with T2DM showed that taking exenatide, a GLP-1 agonist, led to a significant mean weight loss of 3.0 kg and significantly reduced A1C over 30 weeks (117). Another RCT on the effect of liraglutide, another GLP-1 agonist, showed that when taken by individuals with T2DM combined with metformin, liraglutide resulted in a significant reduction in body weight (-1.8 to -2.8 kg depending on dosage) compared to an increase in body weight in the metformin + glimepiride (a sulfonylurea) group (+1.0 kg, p<0.0001).

Hypoglycemia was also reduced in the liraglutide groups and glycemic control was similar compared to the glimepiride group (118). While these results are promising, the cost-effectiveness of these medications for promoting such a small amount of weight loss remains to be seen.

The long-term efficacy of anti-obesity medications remains modest, with mean weight loss of <5 kg after 12 months (119). Safety of these medications remains a primary concern, as the risk of side effects and adverse events with treatment remains high. Pharmacotherapy does not appear to be the solution to long-term weight control, as cessation of treatment often leads to weight regain
and anti-obesity drugs are not approved for use beyond 2 years (2) nor are they recommended for older adults (120). This further underscores the importance of lifestyle approaches to weight management as strategies that can be safely maintained over a lifetime.

2.1.2.2 Surgical Approaches to Weight Loss

The NIH Consensus Statement for Severe Obesity states that the following criteria be used to identify potential surgical candidates: a BMI $\geq 40$ kg/m$^2$ or a BMI $\geq 35$ kg/m$^2$ with co-morbid conditions, including physical conditions that hinder quality of life (10). In 2009, the American Association of Clinical Endocrinologists, the Obesity Society, and the American Society for Metabolic and Bariatric Surgery established clinical practice guidelines for medical management of bariatric surgery patients (121). The expert panel stressed that surgical intervention for obesity treatment must be considered on an individual basis only after other treatment attempts have failed. Care must be taken to ensure the patient understands that surgery is not a replacement for lifestyle changes, and that life-long medical and nutritional follow-up is required after surgical treatment for obesity (121).

An RCT comparing the efficacy of surgery versus lifestyle interventions in obesity treatment demonstrated that although surgery results in greater weight loss after 12 months, similar improvements in risk factors and co-morbidities can be achieved with lifestyle interventions (122). A Swedish study of over 4000 patients with mean follow-up of 10.9 years showed that bariatric surgery resulted in greater weight loss and reduced mortality compared to conventional treatment (123). However, surgical management of obesity is only appropriate for a small subset of the population. A meta-analysis of the efficacy and safety of bariatric surgery estimated that 20% of surgical patients experience adverse events (124). It is also costly, as extensive pre-operative, peri-operative and post-operative evaluation and monitoring of surgical patients by a multidisciplinary team is required (121).
Increased interest in the use of bariatric surgery in the treatment of T2DM has developed due to evidence that gastric bypass and malabsorptive procedures improve fasting blood glucose levels before significant weight loss occurs (125-127). However, treatment guidelines state that preoperative care should focus on optimizing glycemic control via nutrition, physical activity, oral agents and insulin therapy if required (121). As such, surgical management is not a replacement for, but an adjunct to, traditional T2DM therapy and is usually used where conventional treatment has failed. Due to high costs and limited resource availability (128), surgery is not a viable option for many overweight and obese individuals with T2DM.

### 2.1.2.3 Dietary Approaches to Weight Loss

The safest and most widely recommended method to achieve a healthy body weight is lifestyle change to promote negative energy balance, or simply put, to eat less and move more. However, starting an exercise program can be particularly difficult for obese individuals due to physical limitations, joint pain, and high risk of injury due to high body weight and deconditioning from lack of physical activity (129,130). Social stigma can be another barrier to commencing a physical activity program, but once weight loss is initiated through dietary changes, improvements in self-efficacy can increase the motivation of individuals to add exercise to an energy-reduced diet (131). As such, dietary interventions that reduce energy intake are the cornerstone of obesity treatment. To lose weight at a healthy rate of 0.5 to 1 kg/week, an energy deficit of 500 - 1 000 kcal/day is recommended (132). However, consistent restriction of energy intake can be difficult to maintain over the long-term, as feelings of hunger or deprivation can weaken resolve and lead to increases in food intake, resulting in weight regain over time (133-135). Achieving weight loss and keeping the weight off can be incredibly challenging, as evidenced by the multi-billion dollar diet industry.
Fad diets are popular with consumers but controversial with researchers and health care providers, as the weight loss they promote does not tend to be sustained over time. This is often because fad diets work by restricting certain food groups, and these restrictions can be difficult to maintain over an extended period. As a result of these restrictions, fad diets can lack essential nutrients required for overall health. Many fad diets also emphasize a certain ratio of macronutrients as the secret to weight loss. With the exception of reducing saturated fat intake, the current research does not support the effectiveness of manipulating macronutrient distribution for long-term body weight regulation or health (136). An RCT comparing the efficacy of the popular weight loss diets with different macronutrient ratios over 12 months included Atkins (less than 20 grams of carbohydrate per day, slowly increasing to 50 grams per day), Zone (40% of daily energy from carbohydrate, 30% from protein, 30% from fat), Weight Watchers (1200-1500 kcal per day) or Ornish (10% of daily energy from fat, vegetarian diet). No significant difference in weight loss was seen between the groups. Dietary adherence was low for each of the diet plans, with approximately half of the study participants dropping out of each diet group (90). A review of weight loss studies conducted in obese individuals with metabolic syndrome found that diets moderate in protein with most fat in the form of omega-3 and monounsaturated fatty acids (MUFAs) and consisting primarily of low glycemic index (GI) carbohydrates appear to be most beneficial in creating sustainable weight loss when compared to low carbohydrate and low fat diets (136). This suggests that a more balanced approach may be most effective in promoting adherence and result in greater weight loss (137).

Another issue with fad diets is their long-term safety has not been established, and their impact on health and disease prevention is often undocumented (138). Some fad diets have even been shown to be detrimental. For example, restriction of dietary carbohydrate as in the popular Atkins diet has been associated with complications such as lipid abnormalities, heart arrhythmias, cardiac sudden death, osteoporosis and kidney damage (139). The CDA 2013 Clinical Practice
Guidelines emphasize the importance of nutritional adequacy of weight loss diets in this population, and recommend a minimum of 100 g of carbohydrate/day to spare protein degradation, protect against muscle wasting and prevent ketosis (78). Furthermore, evidence suggests that spreading carbohydrate intake evenly throughout the day and maintaining consistency in carbohydrate intake may help control blood glucose and promote a healthy body weight (140,141). As such, it is recommended that overweight and obese individuals adopt balanced dietary patterns known to promote slow and sustainable weight loss and that have been shown to reduce chronic disease risk.

In individuals with T2DM, dietary intake of foods rich in fibre is recommended for promoting weight loss, as fibre consumption has been shown to increase satiety, leading to reduced energy intake (142,143). The CDA advises that individuals with T2DM attempting to lose weight receive counseling from a registered dietitian (RD) on appropriate portion sizes and the selection of nutrient-rich foods such as whole grains and legumes, which are associated with feelings of fullness and subsequent reductions in energy intake (142,144). The literature suggests that weight loss is particularly difficult for individuals with T2DM (145), which further underscores the need for dietary strategies that can help promote negative energy balance.

*Salvia hispanica* seeds are high in fibre and protein, low in available carbohydrates and rich in omega-3 fatty acids and micronutrients, and preliminary evidence suggests they may help promote satiety. These seeds may be useful as part of an energy-reduced diet as they not only increase feelings of fullness, but can offer an important source of nutrients for dieters who are restricting food intake. The nutritional composition of *Salvia hispanica*, combined with its promising effects on satiety and other CVD risk factors from preliminary studies, have highlighted the potential of these seeds for weight management in T2DM.
2.2 *Salvia hispanica* L.

### 2.2.1 Background and Classification

*Salvia hispanica* L. is an oily seed that was used by the Aztecs as both food and medicine. The Aztecs referred to the seeds as “running food” as they provided ample energy during lengthy trading expeditions (146). There are over 80 varieties of *Salvia hispanica*, also known by the common name chia, often varying in its nutritional composition. Through selective breeding, two registered white varieties of the seed were created: Sahi Alba 911 and 912, commercially known as Salba®, developed by Compania Inversora Agropecuaria (Buenos Aires, Argentina).

For simplicity, Salba® will be referred to as Salba from this point forward. Salba seeds are grown in Peru and are commercially available on the Canadian, USA, and New Zealand markets.

### 2.2.2 Nutritional Composition

Preliminary clinical data suggests that Salba may help increase satiety, reduce waist circumference, lower postprandial glycemia, and improve additional CVD risk factors, suggesting its potential role in weight management. The potential health benefits of Salba may be due to its nutritional composition, as research suggests its key nutrients may support body weight regulation (Table 2-1). Salba is a rich source of both soluble and insoluble dietary fibre, a nutrient associated with increased satiety and lower risk of obesity. It also contains all essential amino acids, making it a complete protein. Salba is 21% protein, shown to be one of the most satiating macronutrients. Salba contains 28% fat, with 65% of the fat in the form of alpha-linolenic acid, an omega-3 polyunsaturated fatty acid (n-3) reported to be one of the more satiating types of fat. It is also rich in minerals such as calcium, which may aid in weight management (147). Further, Salba contains antioxidants that may help reduce inflammation (148). Based on the nutritional composition of Salba, it may help to increase satiety and thus support weight loss, which may improve T2DM control and CVD risk factors.
**Table 2-1.** The nutritional composition of *Salvia hispanica* (Salba)*

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Salba (100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>528</td>
</tr>
<tr>
<td>Total Fat (g)</td>
<td></td>
</tr>
<tr>
<td>Omega-3s (g)</td>
<td>32.1</td>
</tr>
<tr>
<td></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></td>
<td>5.6</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>

*Analysis by Maxxam Analytics (2011), Mississauga, ON.*
2.2.2.1 Carbohydrate and Dietary Fibre

Although Salba contains 36.4% total carbohydrate, only 3% is in the form of available carbohydrate and the remainder is dietary fibre. The Institute of Medicine in the U.S. and Health Canada have collaborated to set Dietary Reference Intakes (DRIs), recommended intakes for each nutrient based on age and sex categories of healthy individuals. The Adequate Intake (AI) is the recommended average daily nutrient intake level based on observed or experimentally determined estimate of nutrient intakes by healthy individuals (149). For fibre, the AI is as follows: for ages 9-18: 26 g for females, 31g for males; ages 19-50: 25 g for females, 38 g for males; ages ≥51: 21 g for females, 30 g for males per day (149). Most North Americans fall short of these recommendations, consuming less than 15 g of fibre per day (149). Low fibre intakes may be due to the popularity of carbohydrate-restricted weight loss diets or the higher availability and lower cost of low fibre convenience foods. Increasing fibre intake all at once and without appropriate fluid consumption is also associated with undesirable side effects such as gas, bloating and abdominal discomfort (150). Despite health promotion efforts encouraging increased intake of fibre-rich foods such as vegetables, fruit and whole grains, the general population struggles to meet these goals. As such, it may be necessary to explore incorporating fibre supplements into the North American diet in order to reach AI goals (151).

Dietary fibre can be classified based on various characteristics, including its biology or source, chemical composition, physiological effects such as digestibility, and metabolic effects (152). One of the more common ways to characterize fibres is based on their solubility in a solution similar to the human digestive system (153). Insoluble fibres are those that add bulk to the stool, increase bile acid secretion and reduce intestinal transit time. Soluble fibres increase transit time and are further subdivided based on viscosity, with viscous soluble fibre delaying gastric emptying and slowing the absorption of glucose into the blood (154,155) and nonviscous soluble
fibre acting as a substrate for fermentation by microflora in the colon (156). Of the fibre present in Salba, 16% is highly viscous soluble fibre and the remaining 84% is insoluble fibre.

Dietary fibre has been shown to reduce energy intake by increasing satiety. Several RCTs have shown that high fibre diets or use of fibre supplements causes weight loss (157-160). A meta-analysis of 22 clinical trials demonstrated that a 12 g increase in dietary fibre is associated with a 10% reduction in energy intake and a weight reduction of 1.9 kg over an average study period of 3.8 months (161).

Current evidence suggests that dietary fibre intake may help to reduce levels of low-grade inflammation. A study in women with T2DM examined the associations between long-term intake of bran and other dietary fibers with markers of systemic inflammation. There was a significant trend toward decreasing levels of CRP with increasing quintiles of bran intakes (162).

### 2.2.2.2 Dietary Fat

Salba contains 32.1% fat, with 65% in the form of omega-3 polyunsaturated fatty acids (n-3), 17% as omega-6 polyunsaturated fatty acids (n-6), 6% as monounsaturated fatty acids and 11% as saturated fatty acids. The n-3s found in Salba and other plant sources are in the form of the 18-carbon alpha-linolenic acid (ALA). Conversely, the n-3s found in fatty fish such as salmon, trout and mackerel are the long-chain polyunsaturated fatty acids (PUFAs) eicosapentaneoic acid (EPA) and docosahexaneoic acid (DHA), consisting of 20 and 22 carbons, respectively. Much of the literature supporting the benefits of n-3s for CVD risk reduction has been conducted on EPA and DHA, showing improvements in triglyceride levels in both healthy participants and those with elevated triglyceride levels (163-165). While the body does convert some dietary ALA into EPA and DHA, evidence suggests the conversion rate is poor, estimated at <5% depending on n-6 intake (166). Because n-6s have been shown to have pro-inflammatory effects and n-3s are anti-
inflammatory, the ratio of these fatty acids in the diet is of particular importance for reducing CVD risk. While the recommended n-6:n-3 ratio is 4:1, most Canadians are estimated to consume a ratio as high as 20:1 (167). In order to correct this imbalance, Health Canada recommends that individuals include foods rich in n-3s as part of a healthy eating plan (168).

The effect of long-chain n-3s from fish on glycemic control remains controversial, with several RCTs demonstrating that supplementing with fish oils negatively impacts glycemic control and reduces insulin sensitivity (169,170). A large prospective study provided further support for these findings, demonstrating that risk of T2DM was increased in women with n-3 intakes from fish but not from plant sources (171). A systematic review and meta-analysis of 16 prospective studies on the relationship between n-3 intake and T2DM risk found that there was no effect of fish and/or seafood consumption nor EPA and DHA consumption on risk of developing T2DM. However, dietary ALA was associated with a non-significant trend towards reduced T2DM risk (237). The authors concluded that further studies are needed to investigate the associations between ALA and T2DM (172). As an excellent source of plant-based n-3s, Salba may provide a helpful source of n-3s in the North American diet, especially for vegetarians.

Fat is a concentrated source of kilocalories and improves the mouthfeel and palatability of foods, which can lead to greater energy intake (173,174). Replacing fat with other constituents to create reduced-fat food products therefore often compromises sensory properties. A study of the acceptability, palatability and nutrient composition of cakes with 25% of the fat replaced with Salvia hispanica gel found that no difference in sensory properties was detected compared to the full fat control cakes (175). Potentially, Salba could therefore be used as a more nutritious and energy-reduced substitution to recipes compared to fat alone.

Some evidence suggests that the degree of fatty acid saturation may negatively correlate with satiety. An acute study demonstrated that polyunsaturated fat, the primary type of fat found in
Salba, was shown to increase postprandial satiety compared to saturated and monounsaturated fatty acids as part of a mixed meal (176). Polyunsaturated fatty acids, including n-3s, have been shown to function as transcription factors that influence expression of genes necessary for fat metabolism. N-3s in particular have been shown to promote expression of genes required for fat oxidation and thermogenesis, resulting in reduced fat storage and improvements in glucose clearance (177-179). These findings led to the hypothesis that these acute metabolic effects may help to control weight over time.

The long-term impact of ALA on weight was investigated using a cohort of the European Prospective Investigation into Cancer and Nutrition. Dietary intake data from 25,540 participants was used to evaluate the association between intake of various types of fatty acids and weight change over 6.5 years. Participants with the highest intakes of ALA were shown to have reduced risk of significant weight gain over the follow-up period (180).

Over the long-term, diets that include moderate levels of fat are better adhered to and can lead to greater weight loss than low-fat diets (181), an important consideration when planning dietary interventions for obesity. Based on these findings, both the amount and type of fat found in Salba may help to promote satiety, which may assist in achieving a healthy body weight as part of an energy-reduced diet.

### 2.2.2.3 Protein

Salba consists of 23.1% protein which is comparable to the amount of protein found in lentils (23%) and chickpeas (21%) and higher than other oily seeds (182,183). While several methods are available for evaluating protein quality and digestibility, the position of the WHO and Food and Agriculture Organization (FAO) is that the Protein Digestibility Corrected Amino Acid Score (PDCAAS) be adopted as the international standard for measuring protein values in human
nutrition (184). The PDCAAS for a protein is calculated by expressing the amount of the first limiting essential amino acid of the protein as a percentage of the amount of that amino acid in a reference protein, based on the amino acid requirements of preschool-age children. The value is then corrected for the fecal digestibility of the protein to give the PDCAAS value, truncated to a score of 100% (184). The PDCAAS is not without limitations, including ignoring the effects of ileal digestibility and antinutritional components of foods, as well as differences in digestibility related to age (185). Despite these limitations, it is still widely used and accepted as the preferred method of measuring protein value (186). While a PDCAAS score has not been determined for Salba specifically, the PDCAAS of ground Mexican chia has been reported as 80% (187) due to limiting amounts of lysine compared to requirements of preschool-age children (188). However, Salvia hispanica could still function as an important source of protein when part of a balanced diet containing lysine sources such as pulses (189). By comparison, PDCAAS scores for other commonly consumed foods range from 25% for wheat gluten to 52% for peanuts, 52% for lentils, 92% for beef and 100% for casein and egg whites (186,189). The amino acid composition of Salba is provided in Table 2-2.

Protein is the most satiating macronutrient and its consumption has been shown to increase energy expenditure postprandially (190). One possible mechanism for protein-induced satiety is the increase in the release of the anorexigenic hormone peptide tyrosine tyrosine (PYY) in both lean and obese subjects in response to a protein-rich meal (34). The satiating effects of a high-protein diet given for 1-3 days can result in sustained increases in satiety (191,192), suggesting the usefulness of including protein-rich foods in the planning of hypocaloric diets.

The increase in energy expenditure subsequent to macronutrient ingestion is termed the thermic effect of food (TEF). The TEF is defined as the difference between energy expenditure after consuming a food and resting energy expenditure, divided by the rate of energy intake (193).
Because the macronutrients vary substantially in the amount of energy required for their metabolism and storage, their TEF values differ significantly. The TEF for protein is 20-30%, for carbohydrate is 5-10%, and for fat is 0-3% (193). Further, complete proteins produce greater increases in TEF than incomplete proteins, thus leading to greater energy expenditure (194). Under ad libitum conditions, increasing the protein content of the diet from 10-15% of total energy to 20-30% has been shown to reduce energy intake (194). Studies comparing TEF between lean and obese participants did not show any differences in energy expenditure in response to the macronutrients (195).

Over the long term, higher protein intakes can also improve successful weight maintenance by increasing energy expenditure and also sparing lean body mass (194). The consensus based on the current body of research is that over time periods >1 year, weight loss does not differ between high protein and standard protein diets, as individuals return to more moderate protein intakes (196). As such, the long-term feasibility of following high protein diets is questionable. Nevertheless, incorporating a high protein, tryptophan-rich supplement such as Salba into a hypocaloric diet may be helpful in promoting weight loss over the short term.
Table 2-2. The amino acid composition of Salba

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>mg per 100 g Salba</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>314</td>
</tr>
<tr>
<td>Arginine</td>
<td>518</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>546</td>
</tr>
<tr>
<td>Cysteine</td>
<td>102</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>1080</td>
</tr>
<tr>
<td>Glycine</td>
<td>298</td>
</tr>
<tr>
<td>Histidine</td>
<td>174</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>218</td>
</tr>
<tr>
<td>Leucine</td>
<td>410</td>
</tr>
<tr>
<td>Lysine</td>
<td>288</td>
</tr>
<tr>
<td>Methionine</td>
<td>102</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>312</td>
</tr>
<tr>
<td>Proline</td>
<td>230</td>
</tr>
<tr>
<td>Serine</td>
<td>354</td>
</tr>
<tr>
<td>Threonine</td>
<td>294</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>666</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>142</td>
</tr>
<tr>
<td>Valine</td>
<td>310</td>
</tr>
</tbody>
</table>
2.2.2.4 Minerals

Salba is rich in minerals such as calcium, which has been implicated in the regulation of body weight. Zemel and colleagues, using the NHANES-III data, discovered a strong inverse association between calcium intake and relative risk for obesity (197). Review studies suggest that differences in calcium intake may explain up to 3% of the variance in body weight (198).

Randomized, placebo-controlled, parallel studies have shown an impact of calcium intake on weight loss potential. In an RCT of calcium supplementation in elderly women, the calcium group lost significantly more weight over a 4 year period compared to control (199). Proposed mechanisms for the weight control effect of dietary calcium include less fat accumulation in adipocytes (197,200,201), increased satiety (202), as well as increases in fecal fat excretion leading to a reduction in energy absorption (203). The literature suggests that a 1000 mg difference in calcium intake is associated with an 8 kg difference in mean body weight (198).

Attempts to reduce total energy intake to promote weight loss may lead to inadequate micronutrient intake. As a result, dietitians often recommend multivitamin and mineral supplements to individuals attempting to lose weight. However, selecting whole foods over micronutrient supplements is a cornerstone of dietetic practice (204).

2.2.2.5 Antioxidants

Both T2DM and CVD are associated with oxidative stress caused by hyperglycemic and dyslipidemic states (205). Oxidative stress leads the immune system to respond with chronic inflammation and increases in the production of free radicals, also known as reactive oxygen species (ROS). ROS increase the risk of morbidity and mortality of obesity and T2DM, including increased risk of CVD complications (205,206). The mechanism by which ROS are thought to
increase disease risk is by causing damage to DNA, carbohydrates and proteins, as well as promoting accumulation of oxidized LDL in the blood vessels (207).

Antioxidants are substances that help to mitigate cellular damage by free radicals by accepting an electron from ROS, stabilizing them in the process (208). T2DM has been shown to deplete antioxidant levels in the plasma, which is associated with increased risk of endothelial dysfunction, CAD and CVD (205,206,209). These findings have led to increasing interest in the use of antioxidants in T2DM to improve outcomes.

*Salvia hispanica* is rich in antioxidants such as quercetin, kaempferol, chlorogenic acid and caffeic acid (210). The Total Antioxidant Capacity (TAC), a measure of the ability of an antioxidant to reduce an ROS, has been measured for Salba. At 84 TAC/g, Salba has a higher antioxidant capacity than blueberries, which are often considered to be an excellent source of antioxidants (211). Several large-scale studies have demonstrated negative associations between intakes of antioxidant-rich fruit and vegetables and chronic disease risk in participants with T2DM (212,213). Serum antioxidant levels have also been positively correlated with insulin sensitivity using NHANES data (214). A review of the literature on antioxidants and CVD risk showed that large cohort studies have demonstrated an association between high intakes or high plasma levels of antioxidants and reduced risk of MI or stroke. However, RCTs have not demonstrated an effect of a single antioxidant on CVD event risk (215). As a result, researchers and health care providers recommend that antioxidants be consumed via foods rather than in supplemental form (216).

### 2.2.3 Salba: Past Research

Preliminary preclinical and clinical research suggests the potential benefits of Salba on satiety, waist circumference, glucose control and improvement in CVD risk factors. Taken together, this
literature provides the rationale for investigating the efficacy of Salba seeds in improving weight, glycemc control and reducing CVD risk in overweight and obese individuals with T2DM.

2.2.3.1 Preclinical Studies

A study in male Wistar rats investigated the effect of chia seeds, the black seeds produced by common varieties of *Salvia hispanica*, on plasma lipids (217). Adding chia to the rats’ diet resulted in reductions in TAGs and increases in HDL in the rats’ blood. Furthermore, rats who consumed chia had higher n-3 and lower n-6 levels in their serum. As previously described, a higher ratio of n-3:n-6 is desirable as it has been associated with reduced CVD risk (218).

Another study examined the impact of standardized, white *Salvia hispanica* seeds (Salba) on lipids, visceral fat and IR in rats with metabolic syndrome (219). The researchers induced metabolic syndrome in the rats by feeding them a sucrose-rich diet (SRD) over 3 months which included corn oil as its fat source. Rats were then randomized to either continue on the SRD (the control group) or the SRD with the fat source replaced with Salba seeds for an additional 2 months. The rats consuming Salba showed a significant reduction in visceral adiposity and experienced less weight gain than the control group. Triglyceride levels and IR also improved in the Salba group (219).

2.2.3.2 Clinical Studies

As a follow-up to the promising findings in animal models, several clinical studies were conducted to determine the potential impact of Salba on human health parameters. In a pilot study, 12 healthy individuals consumed up to 50 g of Salba each day over a one month period (220). Salba supplementation appeared to be safe, as safety parameters did not change from baseline and no side effects were reported. Fasting serum TAG levels were reduced and a significant reduction in participants’ DBP was seen, with a mean decrease from $66.1 \pm 8.4$ mmHg
to 61.5 ± 7.0mmHg. Although no change in body weight occurred, waist circumference decreased significantly over the study period (220). Although the study did not have a control group, the findings suggest that Salba may have an impact on abdominal fat and CVD risk factors. This led to further research using Salba in a population at high risk for both abdominal adiposity and microvascular and macrovascular disease: individuals with T2DM.

In a single-blind, placebo-controlled, cross-over study, 20 individuals with well-controlled T2DM (A1C 6.0–8.5%) were randomized to first receive either Salba or wheat bran control for 12 weeks and after a wash-out period, participants received the other supplement for another 12 weeks. Participants incorporated the supplements into their regular diet using the ground form and their supplement baked into study bread matched for energy and fibre (221). The daily dose of supplements was individualized and was calculated at 15 g/1000 kcal of each participant’s estimated energy requirements (EER). Results showed that Salba supplementation did not affect safety parameters, including kidney and liver function and clotting time. Improvements in several CVD risk factors occurred in the Salba group. Intakes of 37g of Salba compared to wheat bran control led to significant reductions in SBP of -6.3±4.2 mmHg from baseline. Von Willebrand factor (vWF) also decreased significantly from baseline in the Salba group, showing a reduction of 21%. Overall inflammation, as measured by CRP, was significantly lower in the Salba group versus control after 12 weeks, leading to a 40% reduction from baseline (221). A1C was also significantly reduced from baseline in the Salba group, but was not significantly different from control. No significant changes from baseline or differences between groups were seen in fasting blood glucose (FBG) and insulin levels. However, participants were already well-controlled before starting the study and continued with medications throughout. As such, any further improvements in glycemic control may be too minor to detect, especially with such a small sample size.
Although body weight was not an outcome measure in this study, participants reported feeling more full after consuming Salba compared to control. This observational finding suggests that Salba may have the potential to increase satiety, warranting further investigation into this seed as a potential weight control aid. The results of this study provided rationale for the current research, as it suggested that Salba may have beneficial effects on CVD risk factors and body weight regulation in individuals with T2DM.

An acute study was conducted in order to investigate the observational findings of satiety with Salba intake mentioned in the previous study and to determine Salba’s short-term impact on postprandial glycemia. A double-blind, crossover RCT was carried out to determine the dose-response effect of Salba on satiety and incremental area under the curve (iAUC) for glucose in healthy individuals. Eleven participants received four types of white bread containing either 0 (control), 7, 14 or 24 g of Salba (low, medium or high doses, respectively). The white bread control was matched to the other breads for available carbohydrates. Subjective satiety ratings were performed using a 100 mm visual analog scale (VAS) and capillary blood glucose samples were taken every 15 minutes over 2 hours. Subjective satiety scores for the high dose showed significant reductions in appetite at 60, 90 and 120 minutes after consuming the bread, at 90 and 120 minutes for the medium dose, and at 120 minutes for the low dose, compared to control. The iAUC appetite ratings decreased by 41, 58 and 63% for the low, medium and high doses compared to control, although these findings did not reach significance (222).

A dose-response reduction in postprandial glycemia (PPG) was seen with all three doses of Salba. The high and medium doses of Salba significantly reduced glucose iAUC by 44% and 25%, respectively (p=0.002). Overall, each gram of Salba appeared to reduce the iAUC for glucose by approximately 2% (222).
Findings from this study suggest that incorporating Salba into white bread may help reduce appetite and potentially lower PPG. These results suggest a potential mechanism that may help explain the findings of the previous long-term study in individuals with T2DM. It is possible that improvements in PPG associated with habitual Salba consumption may lead to reductions in inflammation which in turn may help to reduce BP and coagulation in this population.

A weight loss study was previously conducted using black chia seeds added to the usual diet of 90 overweight individuals (223). Participants were randomized to receive either 25 g of chia or placebo mixed into 250 mL of water immediately before breakfast and dinner, for a total of 50 g of supplement per day over 12 weeks. No significant change in body weight, body composition, blood pressure or markers of inflammation occurred in either of the groups (223). However, the effect of chia seeds on body weight cannot be determined from this study due to several key limitations. Most significantly, participants did not receive counselling on a hypocaloric diet and were instructed to maintain their usual diet during the study. Because 50 g of chia contains approximately 250 kcal, it is possible that participants were consuming even more energy than usual. In order for gradual, steady weight loss of 0.45 – 0.91 kg per week to occur, an energy deficit of at least 500 kcal per day must be achieved (15). By adding the chia seeds to the diet without accounting for the kcal it contains and not changing the overweight participants’ diet patterns to create negative energy balance, it is not surprising that weight loss did not occur.

Another limitation of the study was the timing of administration of the chia seeds. The supplement was taken immediately before meals, without allowing sufficient time for satiating physiological effects to occur. As the findings of the acute dose-response study showed, it takes 60 minutes for significant reductions in subjective appetite scores to occur with a 24 g dose of *Salvia hispanica* (222). Without permitting ample time for satiety to occur after chia consumption, it was unlikely that the supplement would reduce intake at mealtime.
Taken together, the preclinical and clinical research on *Salvia hispanica* to date provides rationale for further investigation into the efficacy and safety of Salba seeds for long-term weight management in a challenging, high-risk population. The current research study will examine whether incorporating Salba seeds into a hypocaloric diet will lead to favourable changes in body weight, body composition, glycemic control and CVD risk factors compared to control in overweight and obese individuals with T2DM.
Chapter 3
Project Overview

3.1 Rationale

The prevalence of obesity in Canada, currently estimated at 1 in 4, is expected to continue to rise each year (224). Obesity poses a significant public health burden, as it significantly increases the risk of chronic diseases such as T2DM (224). Lifestyle interventions, namely dietary strategies, offer the most economic, low-risk and practical solution to promoting the achievement and maintenance of a healthy body weight. Preliminary research suggests that a standardized variety of the oily seeds *Salvia hispanica* L., commercially known as Salba, may help to improve chronic disease risk factors.

Preclinical and clinical studies on Salba have demonstrated its potential for promoting weight control. In rats fed a sucrose-rich diet, Salba consumption led to significant reductions in visceral adiposity and serum triglycerides (219). In healthy human subjects, Salba was shown to reduce waist circumference (220) and in individuals with T2DM, was observed to promote subjective satiety (221). This research points to the potential of Salba as a complement to dietary therapies for obesity. Weight loss is recommended for both prevention and treatment of T2DM, with even modest weight loss leading to improvements in glycemic control and reductions in CVD risk factors. However, weight loss is particularly challenging for individuals with T2DM. As such, investigation into weight loss strategies in T2DM is imperative.

A previous study on the impact of chia seeds, the common variety of black *Salvia hispanica* seeds, on weight loss did not demonstrate an effect (223). However, inherent limitations in the study design prevent conclusions regarding the weight loss impact of *Salvia hispanica* from being drawn (see Section 2.2.3.2). In addition, the impact of Salba on weight has not been determined
in the T2DM population. As such, further research is needed to determine whether Salba may promote safe weight loss as part of an energy-reduced diet in T2DM, as well as its impact on glycemic control and CVD risk factors.

3.2 Objectives

To assess the efficacy of Salba, relative to control, as part of a hypocaloric diet on the following parameters in overweight and obese individuals with T2DM:

*Primary:*
- Body weight

*Secondary:*
- Glycemic control (A1C, fasting glucose and insulin)

*Tertiary:*
- Obesity-related measures (percent body fat [% BF], waist circumference, waist-to-hip ratio [W:H])
- Satiety-related hormones (ghrelin, adiponectin and PYY)
- CVD risk factors
  - Plasma lipids (total cholesterol [TC], low-density lipoprotein [LDL], high-density lipoprotein [HDL], triglycerides [TAGs])
  - Blood pressure (BP)
  - Inflammation (high-sensitivity C-reactive protein [CRP]).

To assess the safety of Salba, relative to control, as part of a hypocaloric diet using the following parameters in overweight and obese individuals with T2DM:

- Kidney function (creatinine [Cr] and urea)
- 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])
• Platelet adhesion (von Willebrand factor [vWF])
• Symptoms (patient-reported records)

3.3 Hypothesis

Based on the promising preclinical and clinical evidence (previously described in Chapter 2), it is expected that compared to control, Salba consumption will result in greater weight loss and improvements in glycemic control and other CVD risk factors and will have no adverse effects on safety parameters or reported symptoms.

Efficacy: Relative to control, Salba consumption as part of a hypocaloric diet in overweight and obese individuals with T2DM will:

Primary Hypothesis: have a greater effect on weight loss

Secondary Hypothesis: have a favourable effect on glycemic control, as measured by improvement of A1C, fasting glucose and insulin

Tertiary Hypothesis: improve additional obesity-related outcomes (%BF, waist circumference, W:H) and CVD risk factors, including blood lipids (with the exception of LDL), BP, and CRP. The greater weight loss associated with Salba will be partially explained by changes in satiety-related hormones (ghrelin, adiponectin and PYY).

Safety: Salba supplementation will not affect safety parameters, as indicated by kidney and liver function, bleeding time and platelet adhesion, and will not be associated with adverse gastrointestinal side effects, as indicated through patient-reported symptoms.
Chapter 4
Materials and Methods

4.1 Study Design

This study followed a randomized, placebo-controlled, double-blind, parallel design in overweight and obese individuals with well-controlled T2DM. The study took place at The Clinical Nutrition and Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada) and was registered in the NIH Clinical Trials database (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% (standard deviation [SD] = 11%) between two parallel groups with the power of 80% at a level of p<0.05, it was estimated that 54 participants per group (108 in total) would be required. Assuming a 22% attrition rate, a total of 132 participants 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 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 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

Eligibility was determined using the participation criteria described hereafter.

**Inclusion Criteria:**
- Individuals with T2DM for at least 1 year treated with diet and/or oral hypoglycemic medications
- A1C between 6.5% and 8.0%
- Between the ages of 35-75 years
- BMI of 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, MI or stroke (within 6 months)
- Blood pressure >160/100 mm Hg
- Consumption of a high fat diet (e.g. excess of 40% of energy from fat)
- 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 orlistat, or other investigational medications)
- Substance abuse, including but not limited to alcohol (>2 drinks a day), nicotine substitutes or regular smoking and marijuana
• Taking supplements of ALA, hemp or flax seeds, dietary fibre, fish oil or consuming n-3-rich 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.

Participants were assigned to consecutive numbers after they provided written informed consent.

4.5 Study Intervention

The study treatment supplements consisted of either ground 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 both given in the form of a powder and were similar in appearance, taste, and odor in order to minimize detectable differences between the treatments and maintain the double-blind study design.

While previous studies on Salba have utilized wheat bran as a control supplement, oat bran was selected for the current study to serve as a positive control. Oat fibre has been proven to lower LDL cholesterol and research shows promise for its potential benefits in weight control, blood glucose regulation and blood pressure reduction (152). For these reasons, it was selected as a barometer against which to rate the efficacy of Salba for weight reduction and glycemic control. Compared to other fibre supplements, oat bran has the additional benefit of being readily available and affordable. Oat bran is also easily baked into bread with a minimal impact on flavour or texture of the final product.
Supplements were provided at a level of 30 g of Salba/1000 kcal intake, or 35.9 g of energy- and fibre-matched control supplement (25.7 g oat bran + 7.1 g inulin + 3.2 g maltodextrin) /1000 kcal 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., where no side effects were reported (220). The Salba and control supplements were portioned into pouches containing the weekly dose for each participant, labelled with unidentifiable codes, by an individual otherwise not involved in the study in order to ensure the blinding of study personnel and participants. Participants were instructed on how to incorporate the study supplements into their diet with the assistance of a recipe book/instruction manual (Appendix 7). Participants 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 participants were provided with a new supply of supplements in a quantity sufficient to last them an additional seven days beyond the next scheduled visit.

Preliminary findings from the early stages of the study indicated that supplement compliance may be compromised over time, as participants reported taste fatigue after several weeks of adding sprinkles to meals (225). In order to minimize monotony and offer another means to take the supplement, a baker was contracted to bake the Salba and control supplement into whole wheat bread. The bread production began halfway through the recruitment period. Care was taken to ensure that only new participants were offered the bread, while participants who had already begun the study were not offered the bread. This was to avoid introducing any source of within-subject variation over the study period.

Participants were advised to consume up to one third of their daily dose of sprinkles as bread, up to a maximum of 2 slices per day. Counselling was provided by the RD to ensure that participants understood that each slice of study bread was considered as a starch choice, and was not to be consumed in addition to the number of starch servings recommended for weight loss on
the individualized meal plan. Participants were also asked to return any unused bread from the previous clinic visit in order to quantify the amount of study bread consumed. The dosage of the study supplement per slice of study bread was multiplied by number of slices consumed and then added to the intake of sprinkles to accurately assess compliance.
Table 4-1. The nutritional composition of the study supplements

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salba</th>
<th>Oat bran-based 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>

Table 4-2. The nutritional composition of the study bread

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Salba bread</th>
<th>Oat bran-based bread</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass per slice (g)</td>
<td>32.6</td>
<td>34.1</td>
</tr>
<tr>
<td>Supplement per slice (g), [%]</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.5 [23.0]</td>
<td>9.0 [26.4]</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>140.1</td>
<td>138.7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>5.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Omega 3 fatty acids (g)</td>
<td>2.7</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>18.7</td>
<td>18.4</td>
</tr>
<tr>
<td>Fibre (g)</td>
<td>7.3</td>
<td>7.3</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>6.2</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Participants were instructed to follow a hypocaloric diet, defined as their individual estimated energy requirements (EER) less 500 kilocalories (kcal) per day, to promote a weight loss of 0.45 – 0.91 kg per week. EER was calculated using the Harrison-Benedict equation multiplied by a “light” or “very light” activity factor of 1.2 or 1.375, as indicated in participants’ physical activity questionnaires (Appendix 5). A minimum caloric intake was set at 1200 kcal/day. Participants met every 2-6 weeks with an RD 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). Dietary counselling with the RD lasted 30 minutes per visit and consisted of comparing food records to the individual’s meal plan. Using motivational interviewing, the RD would encourage the participant to identify areas for improvement and to set goals to work on until the next visit. Participants were encouraged to avoid excessive consumption of high-fat foods, follow portion sizes outlined in the meal plan, and increase their daily intake of fruits and vegetables. Participants were also encouraged to maintain a constant level of physical activity throughout the study period. At each study visit, participants were asked to report on their weekly physical activity including type, frequency, duration and intensity. Each participant was also given a pedometer to record the number of steps taken per day.

4.6 Study Protocol and Timeline

Eligible participants, as assessed by the inclusion and exclusion criteria, were invited to attend the clinic to start the four week long run-in period, prior to which they received instruction from the RD on how to complete their 3-day food records (Appendix 9). During the run-in period, participants 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 ≥2 kg during the 4 week run-in phase were excluded from the study.
For the entire course of the study, participants were advised to stay on their current treatment medication regimen, as prescribed by their family doctor and/or endocrinologist, and to report any changes in their medical status and treatment at each study visit.

During the 24-week treatment phase, participants attended the clinic for follow-up visits at regular intervals for examination, as outlined in Figure 4-1. Participants 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 participants 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, investigators contacted participants in both groups biweekly 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 possibility of adverse side effects with increasing fibre intake, such as bloating, flatulence, constipation and/or diarrhea (150), participants were asked to complete a questionnaire on adverse effects at every visit (Appendix 11).

![Figure 4-1. Study Timeline](image-url)
4.7 Study Measurements

4.7.1 Anthropometric Assessment

At each visit, anthropometric measurements were carried out, including height, weight, body composition by bioelectrical impedance analysis (BIA), waist circumference and hip circumference. 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. Measured height was rounded to the nearest centimeter. After voiding 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 with reported accuracy of ±0.2 kg (226). Percent body fat (%BF) was measured at each visit with the TANITA BC-418 via multifrequency hand-to-foot BIA (227). 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. Waist circumference and hip circumference were measured using a non-stretchable measuring tape following the NIH protocol (228) and were recorded to the nearest centimeter.
Table 4-3. Protocol of measurements conducted at each visit.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-4</td>
</tr>
<tr>
<td><strong>Fasting Blood Sample</strong> (A1C, FBG, fasting insulin, plasma lipids, CRP, safety parameters)</td>
<td>X</td>
</tr>
<tr>
<td><strong>Fasting Blood Sample</strong> (adiponectin, ghrelin, PYY, plasma ALA)</td>
<td>X</td>
</tr>
<tr>
<td><strong>Dual Energy X-Ray Absorptiometry</strong></td>
<td>X</td>
</tr>
<tr>
<td><strong>Office Blood Pressure</strong></td>
<td>X</td>
</tr>
<tr>
<td><strong>Anthropometric Measurements</strong></td>
<td>X</td>
</tr>
<tr>
<td><strong>3-Day Diet Record</strong></td>
<td>X</td>
</tr>
<tr>
<td><strong>Symptoms Diary</strong></td>
<td>X</td>
</tr>
<tr>
<td><strong>Clinical Assessment Questionnaire</strong></td>
<td>X</td>
</tr>
</tbody>
</table>
4.7.2 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, participants 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 analyses.

4.7.3 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 serum separated tubes, EDTA or citrate-treated vacutainer 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.

4.7.3.1 Glycemic Parameters

Whole blood analysis of A1C 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 A1C from HbA. A1C was expressed as a fraction of the total hemoglobin in the sample (229).

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 (230).
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 (231). 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 (231).

### 4.7.3.2 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 (230). Cholesterol oxidase then oxidized free cholesterol, leading to the formation of hydrogen peroxide, which subsequently reacted to produce a coloured quinoneimine product (230). The change in absorbance, measured at 520 nm, was directly proportional to the concentration of TC in the sample (230).

The Beckman SYNCHRON LX System was used to determine the concentration of serum triglycerides (TAGs) by a timed-endpoint method. Lipase was used to hydrolyze TAGs in the sample to glycerol and free fatty acids (FFAs) (230). Three sequential enzymatic reactions with glycerolkinase, glycerophosphate oxidase and horseradish peroxidase then led to the formation of a red quinoneimine dye (230). The change in absorbance, measured at 520 nm, was directly proportional to the concentration of TAGs in the sample (230).

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 (230). The same detergent used for solubilization, also inhibited the reaction of cholesterol enzymes with low density, very-low density, and chylomicron lipoproteins (230). The reagent contained a polyanion that complexed low-density, very low-density and chylomicron lipoproteins and, in doing so, improved the selectivity for HDL (230). The change in absorbance, measure at 560 nm, was directly proportional to the concentration of HDL in the sample (230).

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

\[
\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{TAG}/2.2) \quad (232,233)
\]

This equation is only valid when triglycerides are present at a concentration <4.52 mmol/L (232). Therefore, LDL could not be calculated for TAGs ≥ 4.52 mmol/L.

### 4.7.3.3 Inflammation

Serum high-sensitivity C-Reactive Protein (CRP) was analyzed using the Beckman SYNCHRON LX System via turbidimetry. CRP in combination with a specific antibody formed an insoluble antigen-antibody complex (230). The change in absorbance resulting from the formation of this complex, measured at 340 nm, was proportional to the concentration of CRP in the sample (230).

### 4.7.3.4 Satiety-Related Hormones

Adiponectin, PYY and ghrelin were assessed using the respective Millipore Sandwich Enzyme-linked immunosorbent assay (ELISA) kits. The adiponectin assay is based on concurrent capture of adiponectin molecules from samples to the wells of a microtiter plate coated with a monoclonal anti-human adiponectin antibodies, and binding of a second biotinylated monoclonal anti-human antibody to the captured molecules (234). In the PYY kit, a similar method is employed dependent on the binding of human PYY molecules (both 1~36 and 3~36) by rabbit
anti-human PYY IgG (235), and in the ghrelin assay, the binding of both active and des-octanoyl forms of human ghrelin molecules by anti-human ghrelin IgG occurs, and the plate is coated with anchor cells (236). Additionally, washing of unbound materials from samples, binding of streptavidin-horseradish peroxidase conjugate to the immobilized biotinylated antibodies, washing of excess of free enzyme conjugates, and quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3′,5,5′-tetramethylbenzidine is evaluated (234-236). The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm – 590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured adiponectin, PYY or ghrelin in the unknown sample, the latter is determined by using a reference curve generated in the same assay with reference standards of known concentrations of human adiponectin, PYY and ghrelin, respectively (234-236). To maximize the protection of active ghrelin within the samples, collection tubes without anticoagulant were used, 4-(2-Aminoethyl) benzenesulfonyl fluoride (AEBSF) was immediately added, and acidification of the serum was achieved using HCl to a final concentration of 0.05N. Research suggests that 20-60% of ghrelin content will be lost if this treatment is not carried out (236).

4.7.4 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-ketoglutarate to oxaloacetate and L-glutamate. Subsequently, oxaloacetate was reduced to malate, while β-Nicotinamide Adenine Dinucleotide (NADH) was simultaneously oxidized to NAD+ (230). The rate of change in absorbance, measured at 340nm, was directly proportional to the activity of AST (230). 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 (230). 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 (230).

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 (230).

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 (237). Factor XII was activated using a phospholipid reagent composed of lipids and an activator reagent (237).

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 (238). This resulted in the conversion of fibrinogen to fibrin and the subsequent formation of a solid gel (238). The time required for clot formation was measured as PT (238).

The International Normalized Ratio (238) was calculated from PT and mean PT normal range of a control sample according to the following formula:

\[
\text{INR} = \frac{\text{PT}_{\text{test}}}{\text{ ISI }} \quad \frac{\text{PT}_{\text{normal}}}{\text{PT}_{\text{normal}}}
\]  

(238)
Where ISI is the International Sensitivity Index based on the tissue factor used to activate the reaction (238).

Von Willebrand Factor (vWF) was measured using venous blood samples drawn from participants’ antecubital vein into vacutainer tubes containing the anticoagulant trisodium citrate (3.2%). A quantitative direct enzyme immunoassay (EMD Millipore, Canada) was used to determine vWF activity. Plasma was separated by centrifuge over 10 min at an RPM of 2500 × g. Plasma (239) was placed into the wells of microtitre strips coated with purified murine anti-vWF immunoglobulin G monoclonal antibody (240). After the first incubation, the wells were washed to remove unbound plasma. A horseradish-peroxidase-labelled mouse anti-human monoclonal anti-vWF conjugate was bound to surface-associated antibody during the second incubation. After another washing step, specifically bound antibody was treated with substrate solution (240). The amount of bound conjugate was given in absorbance units measured with an absorption photometer. The vWF activity was then estimated using the dose-response curve given in the 4th International Standard for Factor VIII and von Willebrand factor in Plasma (241).

4.7.5 Compliance

4.7.5.1 Supplement Consumption

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

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

Where \( x \) was the number of days of treatment.
4.7.5.2 Diet Analysis

Participants 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). The Canadian Nutrient File 2010 version data (Health Canada, Ottawa, Canada) was used for each food item. If this data was not available for a food, the USDA data was used. An average of the 3-day diet profile was generated for the analysis. Diets were analyzed for total energy, macronutrient and calcium 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 was tested for normality using the Shapiro-Wilk test. For variables for which normality was rejected, non-parametric tests were conducted and their p values reported. Subject characteristics were expressed as mean±standard deviation (SD), while all other data was presented as mean±standard error of the mean (SE).

To calculate percent change from baseline for each variable, ANCOVA was used to test significance and results were then converted to percent change using mean baseline and post-treatment scores (242). Comparison of differences from baseline to treatment-end in all parameters of efficacy, safety and compliance were assessed within and between treatment arms using GLM repeated measures ANCOVA. To correct for non-circularity in the covariance matrix, the Geisser-Greenhouse adjustment was used (243). Between treatment middle and end results were adjusted for baseline values and all comparisons were adjusted for age, sex, BMI and medication use. For satiety-related hormones, results were adjusted for baseline values, net weight change from baseline, BMI and for other potential confounders identified in the literature.
These included BF% and insulin levels for ghrelin (22), fasting TAGs and sex for adiponectin (28,29) and waist circumference for PYY (36). Data was considered statistically significant at \( p<0.05 \). For variables found to be significant, post-hoc testing was conducted via the Tukey-Kramer multiple comparison test for between-group differences and Bonferroni for within-group comparisons.
Chapter 5
Results

5.1 Study Participants

Of the 419 individuals contacted by The Clinical Nutrition and Risk Factor Modification Centre at St. Michael’s Hospital, 357 were telephone screened. From the 357 individuals that were telephone screened, 180 attended an information session, 102 of whom expressed further interest in participating in the study and provided informed consent. Out of the 102 subjects who were enrolled in the study and underwent a subsequent clinical screening visit, 24 did not meet further eligibility requirements.

From the 78 subjects who met all of the eligibility requirements, 9 subjects were unable to make the time commitment, 6 subjects were lost to follow-up, 4 withdrew because of unrelated illness, 3 withdrew due to undesired side effects from the study material and 1 moved away. As a result of low supplement compliance (<50%), the results from one subject in the Salba treatment group were excluded from the analysis as per the study protocol. Furthermore, one of the participants in the control group underwent major surgery prior to week 12. Due to the substantial impact on dietary intake and probable effect on body weight, this subject was excluded from the final analysis. Of the 54 subjects who completed the entire 24 week study protocol, 26 were in the Salba treatment group and 28 were in the control group.

To avoid bias that may have resulted from omitting data from participants who had completed more than half of the study protocol, subjects who completed up to week 18 were included in the final analysis. Missing variables for these participants were filled in using last value carried forward. Of the 10 dropouts/excluded participants in the control group, 7 occurred before week 18 and 3 occurred after week 18. In the Salba group, 12 participants dropped out or were
excluded before week 18 and 1 occurred after week 18. In summary, this meant that results from 3 participants were added to the control group and 1 additional participant was included in the treatment group. Therefore, the data from 4 participants was added to the 54 subjects who completed the entire 24-week study protocol. The addition of the week 18 completers to the treatment and control groups is shown in Figure 5-1.

Data herein are present for 31 subjects in the control group and 27 in the treatment group, for a total sample size of 58, unless otherwise indicated. Baseline subject characteristics are presented in Table 5-1.
Figure 5-1. Recruitment flowchart, with inclusion of participants who completed at least week 18 of the study protocol.
Table 5-1. Baseline subject characteristics, presented as mean±SD.

<table>
<thead>
<tr>
<th>Subject Characteristic</th>
<th>Treatment</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salba</td>
<td>Oat Bran</td>
</tr>
<tr>
<td>Sample size (n)</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Sex (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.0±1.6</td>
<td>60.1±1.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.1±2.6</td>
<td>84.2±2.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.0±0.9</td>
<td>30.7±0.7</td>
</tr>
<tr>
<td>BF (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXA</td>
<td>43.9±1.5</td>
<td>42.0±7.7</td>
</tr>
<tr>
<td>BIA</td>
<td>37.2±1.4</td>
<td>38.9±1.4</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>103.5±3.5</td>
<td>104.6±3.0</td>
</tr>
<tr>
<td>Female</td>
<td>104.9±2.5</td>
<td>102.2±1.9</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>125.0±2.4</td>
<td>122.0±2.2</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>72.8±1.4</td>
<td>72.7±1.6</td>
</tr>
<tr>
<td>Medication Use (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AHA</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>LLA</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>AHTN</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>6.8±0.2</td>
<td>7.0±0.2</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>7.4±0.3</td>
<td>7.6±0.3</td>
</tr>
</tbody>
</table>

NS: Not significant (p>0.05) via two sample T-test assuming equal variances. Variance between the two groups assumed to be equal via F-test 2 sample for variance.

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 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), direct renin inhibitors (Rasilez), β-blockers (Atenolol, Bisoprolol), α-adrenergic receptor agonists (Apo-Methyldopa) and combinations of these (Hyzaar, Avalide). Cholesterol medications used were statins (Crestor, Lipitor, Lescol). Four participants in the Salba treatment group and 4 participants in the control group were not taking any medications.

Anthropometric measures of percent body fat (%BF), waist circumference and body mass index (BMI) were also similar between the groups.

5.2 Treatment of Missing Data

Missing values at baseline (Week 0) or end of treatment (Week 24) were recorded as intermediate values (Week 12). Missing values during the intermediate visit were calculated using an average of baseline and end of treatment values. In addition, 3 participants were missing LDL values. These could not be calculated with the algorithm used owing to TAG levels that were ≥ 4.52 mmol/L. As such, LDL results are presented for n=55. Participants who did not attend the final study visit (Week 24) had values completed using the last value carried forward. This was done for 3 participants in the oat bran group and 1 participant in the Salba group. Measurements such as satiety-related hormones and %BF measured by DXA were only collected at baseline and the end of the study, so that participants who did not attend the final study visit (n=4) had missing
values for these variables. Because intermediate measures were not collected, last result carried forward could not be applied for these variables. As a result, data for satiety-related hormones is presented for n=54. As DXA scans were also conducted only at baseline and end of treatment, participants who dropped out before the week 24 visit (n=4) did not have DXA results included in the final analysis. In addition, 3 participants declined the DXA test due to concerns about radiation exposure, despite assurances from the study investigators that exposure would be lower than when travelling from Toronto to Vancouver on an airplane (244). As such, DXA results are presented for n=51.

5.3 Efficacy of Salba

5.3.1 Effect on Primary Outcome Measure

5.3.1.1 Body Weight

The analysis of weight change from baseline resulted in weight loss of 1.4±0.4 kg in the Salba group (n=27) and 0.8±0.3 kg in the oat bran group (n=31) at 12 weeks (NS). At week 18, participants in the Salba group lost 1.4±0.4 kg which was significantly greater than the weight loss of 0.4±0.4 kg in the oat bran group (p=0.045). At week 24, participants in the Salba group lost 1.8±0.5 kg from baseline which was significantly more than the weight loss of 0.5±0.4 kg in the oat bran group (p=0.039). Net changes from baseline are shown in Figure 5-2.

Within the Salba group, weight was significantly different from baseline at weeks 12, 18 and 24 but no significant within-group differences were seen for the oat bran group. A significant interaction between treatment and time was determined using a repeated measures ANCOVA, indicating that mean weight between treatment groups differed over time (p=0.020). Absolute values (mean±SE) and % change are provided in Table 5-2.
Table 5-2. The effect of Salba, compared to oat bran-based control, on body weight, waist and hip circumference and % body fat measured by bioelectrical impedance at middle and end of treatment compared to baseline. For all parameters n=58.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12 (% Change)</td>
<td>Week 24 (% Change)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.1±2.8 (^a)</td>
<td>82.5±0.4 (^b) (-1.9±3.3)</td>
<td>82.2±0.5 (^b) (-2.3±3.3)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>104.6±1.9 (^a)</td>
<td>102.3±0.6 (^ab) (-2.2±1.9)</td>
<td>100.2±0.8 (^b) (-4.2±1.9)</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>111.6±1.9 (^a)</td>
<td>108.7±0.5 (^b) (-2.6±1.7)</td>
<td>108.5±0.8 (^b) (-2.8±1.8)</td>
</tr>
<tr>
<td>W:H</td>
<td>0.94±0.01</td>
<td>0.94±0.005 (0.0±1.2)</td>
<td>0.93±0.007 (-1.1±1.3)</td>
</tr>
<tr>
<td>%BF BIA</td>
<td>38.9±1.6</td>
<td>37.5±0.4 (-3.6±4.1)</td>
<td>37.6±0.5 (-3.3±4.2)</td>
</tr>
</tbody>
</table>

Data are mean±SE. P values by GLM repeated measures ANCOVA adjusted for age, sex, BMI and starting values. Values with different superscripts indicate significant difference at p<0.05. % Change calculated from baseline.

%BF – body fat percentage; BIA -bioelectrical impedance analysis; W:H – ratio of waist:hip circumference.
Figure 5-2. The effect of Salba versus oat bran-based control on change in body weight from baseline, n=58. Results are presented as means with the SE indicated by the vertical lines. * indicates p = 0.045 and ** indicates p = 0.039.
5.3.2 Effect on Secondary Outcome Measures

5.3.2.1 Glycated Hemoglobin, Fasting Blood Glucose and Insulin

There were no significant differences in measures of glycemic control (A1C, fasting glucose and fasting insulin) between or within the groups over time. FBG was approximately 6% lower than baseline after 24 weeks on Salba compared to 3% lower in the oat bran group, but the differences in change from baseline between groups were not statistically significant (p=0.238). See Table 5-3 and Figures 5-3, 5-4 and 5-5.
Table 5-3. The effect of Salba, compared to oat bran-based control, on fasting blood glucose, glycated hemoglobin and fasting insulin at middle and end of treatment compared to baseline. For all parameters n=58.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
</tr>
<tr>
<td></td>
<td>(% Change)</td>
<td>(% Change)</td>
<td>(% Change)</td>
</tr>
<tr>
<td>Fasting Glucose</td>
<td>6.8±0.3</td>
<td>6.7±0.4</td>
<td>6.4±0.4</td>
</tr>
<tr>
<td>(mmol/L)</td>
<td>(-1.5±0.4)</td>
<td>(-5.6±0.4)</td>
<td>(-4.0±0.3)</td>
</tr>
<tr>
<td>A1C (%)</td>
<td>6.6±0.2</td>
<td>6.4±0.1</td>
<td>6.5±0.1</td>
</tr>
<tr>
<td></td>
<td>(-3.0±0.1)</td>
<td>(-1.5±0.1)</td>
<td>(-4.3±0.2)</td>
</tr>
<tr>
<td>Fasting Insulin</td>
<td>71.3±8.2</td>
<td>72.1±4.6</td>
<td>69.6±5.3</td>
</tr>
<tr>
<td>(pmol/L)</td>
<td>(1.1±6.8)</td>
<td>(2.4±7.2)</td>
<td>(-1.8±12.3)</td>
</tr>
</tbody>
</table>

Data are mean±SE. P by GLM repeated measures ANCOVA adjusted for age, sex, BMI, AHAs, change in weight and baseline values. % change calculated from baseline.

A1C – glycated hemoglobin.
Figure 5-3. The effect of Salba compared to oat bran-based control on change in glycated hemoglobin at middle and end of treatment compared to baseline, n=58. Results are presented as mean±SE.
Figure 5-4. The effect of Salba compared to oat bran-based control on change in fasting blood glucose levels at middle and end of treatment compared to baseline, n=58. Results are presented as mean±SE.
**Figure 5-5.** The effect of Salba compared to oat bran-based control on change in fasting serum insulin levels at middle and end of treatment compared to baseline, n=58. Results are presented as mean±SE.
5.3.3 Effect on Tertiary Outcome Measures

5.3.3.1 Obesity-Related Outcome Measures

5.3.3.1.1 Percent Body Fat

No significant differences between or within groups were observed in %BF measured by BIA. See Table 5-2.

When measured using DXA, %BF also did not change between or within groups (see Table 5-4). Upon examining %BF by region, the change in the percentage of android fat (located in the abdominal region) and gynoid fat (located around the hips and thighs) from baseline was not significantly different between the groups. However, % android fat and % gynoid fat changed significantly from baseline within the Salba group (p= 0.031 and p= 0.047, respectively) but not in the oat bran group after 24 weeks.

The ratio of % android to % gynoid fat also did not change significantly between or within groups. See Table 5-4 and Figure 5-6.

DXA measurements were also used to quantify changes in fat mass and fat-free mass. Both groups appeared to lose a small amount of fat from baseline (NS), but no significant differences were seen between the Salba and oat bran groups. See Figure 5-7.
**Table 5-4.** The effect of Salba, compared to oat bran-based control, on total body fat percentage and regional fat distribution as measured by dual energy x-ray absorptiometry at middle and end of treatment compared to baseline. For all parameters n=51.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=24)</th>
<th>Oat Bran (n=27)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 24 (% Change)</td>
<td>Week 0</td>
</tr>
<tr>
<td>Total %BF</td>
<td>44.0±1.7</td>
<td>41.9±0.4</td>
<td>41.2±1.6</td>
</tr>
<tr>
<td></td>
<td>(-4.8±3.8)</td>
<td></td>
<td>(2.0±4.1)</td>
</tr>
<tr>
<td>% Android</td>
<td>48.8±1.3 (^a)</td>
<td>47.0±0.5 (^b)</td>
<td>47.8±1.2</td>
</tr>
<tr>
<td></td>
<td>(-3.7±2.8)</td>
<td></td>
<td>(0.0±2.7)</td>
</tr>
<tr>
<td>% Gynoid</td>
<td>47.8±2.0 (^a)</td>
<td>44.5±0.3 (^b)</td>
<td>43.1±1.9</td>
</tr>
<tr>
<td></td>
<td>(-6.9±3.9)</td>
<td></td>
<td>(4.4±4.7)</td>
</tr>
<tr>
<td>A:G</td>
<td>1.0±0.04</td>
<td>1.1±0.008</td>
<td>1.1±0.03</td>
</tr>
<tr>
<td></td>
<td>(10.0±4.5)</td>
<td></td>
<td>(0.0±2.8)</td>
</tr>
</tbody>
</table>

Data are mean±SE. P values by GLM repeated measures ANCOVA adjusted for age, sex, BMI and starting values. Values with different superscripts indicate significance difference at p<0.05. % change calculated from baseline.

DXA - Dual energy x-ray absorptiometry; A:G- % android body fat to % gynoid body fat ratio.
Figure 5-6. The effect of Salba compared to oat bran on change from baseline in total %BF, %BF in the android region and %BF in the gynoid region as measured by DXA, n=51. Results are presented as mean±SE. * indicates significant within-group change from baseline, p<0.05.
Figure 5-7. The effect of Salba compared to oat bran on change from baseline in total body weight, lean mass and fat mass in kilograms as measured by DXA, n=51. Results are presented as mean±SE. * indicates significant change from baseline within and between groups, p<0.05.
5.3.3.1.2 Waist and Hip Circumference

The analysis of change in waist circumference from baseline showed a trend towards a reduction of \(2.1\pm0.6\) cm in the Salba group (\(n=27\)) vs. \(2.0\pm0.6\) cm in the oat bran group (\(n=31\)) at 12 weeks (NS). At week 18, waist circumference in the Salba group was reduced by \(3.2\pm0.8\) cm which was also not significantly different than the reduction of \(1.6\pm0.8\) cm in the oat bran group (\(p=0.184\)). At week 24, waist circumference was significantly reduced in the Salba group by \(3.5\pm0.7\) cm compared to oat bran control with a reduction of \(1.1\pm0.7\) cm from baseline (\(p=0.021\)). Net changes from baseline are shown in Figure 5-8.

Within the Salba group, waist circumference changed significantly from baseline by weeks 18 and 24. No significant change in waist circumference from baseline occurred in the oat bran group. A significant interaction between treatment and time was determined using a repeated measures ANCOVA, indicating that mean waist circumference between treatment groups differed over time (\(p=0.027\)). Absolute values (mean±SE) and % change are provided in Table 5-2.

Statistical analyses of changes in hip circumference from baseline did not reveal significant differences between groups at any time point. However, hip circumference changed significantly from baseline in the Salba group at weeks 12, 18 and 24. No significant changes from baseline occurred in the oat bran group. See Table 5-2.

Waist to hip ratio was also not significantly different between groups. No significant difference was observed in comparing change from baseline in either group at any of the time points. See Table 5-2.
Figure 5-8. The effect of Salba versus oat bran on change in waist circumference from baseline, n=58. Results are presented as mean±SE. * indicates p= 0.021.
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 within or between the treatment groups. See Table 5-5.

5.3.3.2.2 Lipid Parameters

No significant differences were seen when comparing the mean change from baseline in the Salba or in the oat bran groups for TC, TAGs, HDL, HDL-C, LDL or vWF. Baseline lipids were within targets established by the 2008 Clinical Practice Guidelines with the exception of LDL levels which were marginally elevated (65). See Table 5-6.

CRP was not significantly different from baseline or between groups at week 12. At week 24, the analysis of change in CRP from baseline showed a significant reduction in the Salba group of 1.41±0.5 mg/L compared to a reduction of 0.2±0.4 mg/L in the oat bran group (p=0.020). Within the Salba group, CRP changed significantly from baseline by week 24. No significant change from baseline occurred in the oat bran group. Net changes from baseline are shown in Figure 5-9.

A significant interaction between treatment and time for CRP was determined using a repeated measures ANCOVA, indicating that mean CRP between treatment groups differed over time (p=0.045). Absolute values (mean±SE) and % change are provided in Table 5-6.
Table 5-5. The effect of Salba, compared to oat bran-based control, on office blood pressure at middle and end of treatment compared to baseline. For all parameters n=58.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12 (% Change)</td>
<td>Week 24 (% Change)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>122.0±2.4 (2.1±2.8)</td>
<td>124.6±2.4</td>
<td>122.7±2.2 (0.6±2.7)</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>72.7±1.6 (-0.3±2.8)</td>
<td>72.5±1.3</td>
<td>72.2±1.3 (-0.7±2.8)</td>
</tr>
</tbody>
</table>

Data are mean±SE. P values by GLM repeated measures ANCOVA adjusted for age, sex, BMI, starting values, change in body weight and blood pressure medications. % change calculated from baseline.

SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure.
Table 5-6. The effect of Salba, compared to oat bran-based control, on fasting serum lipids and inflammation at middle and end of treatment compared to baseline. For all parameters n=58, with the exception of LDL (n=55).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12 (% Change)</td>
<td>Week 24 (% Change)</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.6±0.2</td>
<td>4.4±0.1 (-4.3±4.7)</td>
<td>4.5±0.1 (-2.2±4.8)</td>
</tr>
<tr>
<td>TAGs (mmol/L)</td>
<td>1.5±0.2</td>
<td>1.3±0.1 (-13.3±13.3)</td>
<td>1.4±0.1 (-6.7±14.1)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3±0.06</td>
<td>1.3±0.03 (0.0±5.2)</td>
<td>1.3±0.03 (0.0±5.2)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>3.7±0.2</td>
<td>3.6±0.1 (-2.7±5.9)</td>
<td>3.6±0.2 (-2.7±7.5)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.6±0.2</td>
<td>2.5±0.1 (-3.8±8.3)</td>
<td>2.5±0.1 (-3.8±8.3)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>2.8±0.6 a</td>
<td>2.3±0.7 a (-17.9±24.7)</td>
<td>1.7±0.5 b (-39.3±17.1)</td>
</tr>
</tbody>
</table>

*n applies to all variables except LDL, where n=26 for the Salba group and n=29 for oat bran. All data are mean±SE. P values by GLM repeated measures ANCOVA adjusted for age, sex, BMI, starting values, change in body weight and lipid medications. Values with different superscripts
indicate significance difference at p<0.05. % change calculated from baseline. TC- Total Cholesterol; HDL- High-Density Lipoprotein; HDL-C – TC to HDL Ratio; LDL- Low-Density Lipoprotein; TAGs – Triglycerides; CRP – High-sensitivity C-reactive Protein.
Figure 5-9. The effect of Salba, compared to oat bran-based control, on change in fasting high-sensitivity C-reactive protein levels at middle and end of treatment compared to baseline, n=58. Results are presented as mean±SE. *indicates p=0.020.
5.3.3.3 Satiety-Related Hormones

After adjusting baseline ghrelin levels for BMI, body fat and insulin levels (22), week 0 values were significantly different between groups (p=0.027). Controlling for the same confounding variables, in addition to baseline values and change in body weight, change in ghrelin levels at week 24 was not significantly different between groups. Within the Salba group, ghrelin levels changed significantly from baseline for a reduction of 17% at week 24 (p=0.039). While ghrelin levels appeared to increase from baseline in the oat bran group by approximately 20%, this within-group change approached but did not reach significance (p=0.094).

After adjusting for baseline values, weight change, BMI, fasting TAGs and sex (28,29), change in adiponectin levels by week 24 was found to be significantly greater in the Salba group compared to the oat bran group (p=0.022). Adiponectin levels within the Salba group also changed significantly from baseline for an increase of approximately 7% (p=0.048). At week 24, adiponectin levels did not change from baseline within the oat bran group.

PYY levels were adjusted for BMI, waist circumference, change in body weight and baseline values (36). No significant changes between or within groups occurred over the study period for PYY. Net changes from baseline are shown in Figure 5-10. Absolute values (mean±SE) and % change are provided in Table 5-7.
Table 5-7. The effect of Salba, compared to oat bran-based control, on fasting ghrelin, adiponectin and PYY levels at middle and end of treatment compared to baseline. For all parameters n=54.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=26)</th>
<th>Oat Bran (n=28)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 24</td>
<td>Change %</td>
</tr>
<tr>
<td>Ghrelin (pg/mL)</td>
<td>676.6±63.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>561.1±25.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-17.1±8.7&lt;sup&gt;δ&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>7.7±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.2±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.5±0.7&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>PYY (pg/ml)</td>
<td>165.7±10.7</td>
<td>158.2±8.3</td>
<td>-4.5±9.8</td>
</tr>
</tbody>
</table>

All values are mean±SE. P values from GLM ANCOVA. Values with different superscripts indicate significant difference at p<0.05. δ indicates p=0.039. † indicates p=0.048.

PYY- Peptide Tyrosine Tyrosine.
Figure 5-10. The effect of Salba (■) compared to oat bran-based control (■) on change in fasting ghrelin, adiponectin and PYY levels from baseline to week 24, n=54. Results are presented as mean±SE.*indicates p=0.022 between groups. †indicates p<0.05 within-group change from baseline.
5.3.4 Effect on Safety Parameters

5.3.4.1 Kidney & Liver Function, Bleeding Time and Platelet Adhesion

Mean measures of kidney function (urea and creatinine) and liver function (AST, ALP, ALT) did not significantly differ between or within groups at any time point. INR, PT and APTT, measures of bleeding time, and vWF, a measure of platelet adhesion, were not significantly different. See Table 5-8. Baseline, week 12 and week 24 values were within normal ranges as set by St. Michael’s Hospital Core Laboratory.
Table 5-8. The effect of Salba, compared to oat bran-based control, on kidney and liver function, bleeding time and platelet adhesion at middle and end of treatment compared to baseline. For all parameters n=58.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
<td>Week 24</td>
</tr>
<tr>
<td><strong>Urea (mmol/L)</strong></td>
<td>6.0±0.4</td>
<td>5.9±0.2</td>
<td>5.7±0.2</td>
</tr>
<tr>
<td><strong>Cr (umol/L)</strong></td>
<td>74.0±3.3</td>
<td>75.0±1.1</td>
<td>74.0±1.4</td>
</tr>
<tr>
<td><strong>AST (U/L)</strong></td>
<td>24.6±1.8</td>
<td>22.1±0.9</td>
<td>23.9±1.8</td>
</tr>
<tr>
<td><strong>ALP (U/L)</strong></td>
<td>69.7±4.7</td>
<td>68.4±1.9</td>
<td>67.3±2.7</td>
</tr>
<tr>
<td><strong>ALT (U/L)</strong></td>
<td>25.6±2.8</td>
<td>24.9±1.6</td>
<td>25.6±1.6</td>
</tr>
<tr>
<td><strong>PT (s)</strong></td>
<td>11.1±0.1</td>
<td>11.0±0.1</td>
<td>10.9±0.1</td>
</tr>
<tr>
<td><strong>INR (s)</strong></td>
<td>1.0±0.01</td>
<td>1.0±0.01</td>
<td>1.0±0.01</td>
</tr>
<tr>
<td><strong>APTT (s)</strong></td>
<td>31.4±0.6</td>
<td>30.1±0.4</td>
<td>30.9±0.4</td>
</tr>
<tr>
<td><strong>vWF (IU/mL)</strong></td>
<td>1.6±0.1</td>
<td>1.6±0.06</td>
<td>1.6±0.06</td>
</tr>
</tbody>
</table>

Data are mean±SE. P value by GLM repeated measures ANCOVA adjusted for age, sex, weight, aspirin use and baseline values. Values with different superscripts indicate significant difference at p<0.05.

Cr- Creatinine; AST- Aspartate Aminotransferase; ALP- Alkaline Phosphatase; ALT- Alanine Amino Transferase; PT- Prothrombin Time; INR- International Normalized Ratio; APTT – Activated Partial Thromboplastin Time; vWF – von Willebrand Factor.
5.3.4.2 Reported Symptoms

Presented in Table 5-9 are the symptoms that were documented by participants at baseline and during the middle and end of the treatment phase of the study. Reported side effects did not change significantly during the treatment period in either group.

Table 5-9. The number of participants who reported symptoms at baseline, middle and end of the study in each of the treatment groups*.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</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>2</td>
<td>1</td>
</tr>
<tr>
<td>Constipation (n)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Excessive Urination (n)</td>
<td>1</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>
<tr>
<td>Any Symptom (n)</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

*All changes between and within groups over time NS as determined by p>0.05 by GLM ANCOVA.
5.3.5 Compliance

5.3.5.1 Supplement Consumption

Reported supplement consumption was not significantly different between the groups and did not change significantly over time. Participants in the Salba treatment group were prescribed (mean±SD) 39.8±1.3 g of Salba to consume daily. Participants in the control group were prescribed 48.7±1.7 g of the energy- and fibre-matched oat bran-based supplement per day.

Supplement compliance (mean±SE) for the first 12 weeks of the treatment phase was 94.1±6.0 % for the Salba group and 83.6±6.3 % for the oat bran group. For the second half of the study, supplement compliance was 84.7±4.5 % for the Salba group and 81.9±6.9% for the oat bran group. See Table 5-10.

Plasma phospholipid fatty acid levels were also compared at the end of the study period to determine whether there was a difference in n-3 levels between groups. ALA levels were significantly higher in the Salba group versus the oat bran group (p = 7.5 x 10⁻⁶). Polyunsaturated fatty acids linoleic acid and arachidonic acid were also found to be significantly different between groups, with the former being higher in the Salba group (p=0.0055) and the latter higher in the oat bran group (p=0.035). However, total n-6 did not differ between groups. See Table 5-11.
**Table 5.10.** Comparison of supplement consumption as reported by study participants to prescribed supplement amount during the first half of the study period, the second half of the study period and overall in the Salba and oat bran groups. For all parameters n=58.

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Salba (n=27)</th>
<th></th>
<th></th>
<th></th>
<th>Oat Bran (n=31)</th>
<th></th>
<th></th>
<th></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></td>
<td>Week 0-12</td>
<td>Week 12-24</td>
<td>Week 0-24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prescribed Amount (g)</td>
<td>39.8±1.3</td>
<td>39.8±1.3</td>
<td>39.8±1.3</td>
<td>NS</td>
<td>48.7±1.7</td>
<td>48.7±1.7</td>
<td>48.7±1.7</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Consumed Amount (g)</td>
<td>36.7±1.3</td>
<td>33.7±1.1</td>
<td>35.2±1.2</td>
<td>NS</td>
<td>40.7±2.2</td>
<td>39.9±2.1</td>
<td>40.0±1.3</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Data are mean±SE. NS indicates non-significance determined by *p* > 0.05 by GLM ANCOVA.
Table 5-11. Comparison of plasma phospholipid fatty acid content between treatment groups at the end of the study. For all parameters n=54.

<table>
<thead>
<tr>
<th>Fatty Acids (nmol/L serum)</th>
<th>Salba (n=26)</th>
<th>Oat Bran (n=28)</th>
<th>P value between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum SFA†</td>
<td>46.0±0.5</td>
<td>46.8±0.4</td>
<td>NS</td>
</tr>
<tr>
<td>Sum MUFA†</td>
<td>19.2±0.5</td>
<td>19.9±0.5</td>
<td>NS</td>
</tr>
<tr>
<td>ALA 18:3n-3</td>
<td>1.03±0.11*</td>
<td>0.5±0.03</td>
<td>7.5 x 10⁻⁶</td>
</tr>
<tr>
<td>EPA 20:5n-3</td>
<td>1.6±0.2</td>
<td>1.9±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>DHA 22:6n-3</td>
<td>2.2±0.1</td>
<td>2.3±0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Sum n-3 PUFA†</td>
<td>6.9±0.3</td>
<td>6.7±0.3</td>
<td>NS</td>
</tr>
<tr>
<td>LA (18:2n-6)</td>
<td>21.1±0.6*</td>
<td>18.8±0.5</td>
<td>0.0055</td>
</tr>
<tr>
<td>ARA (20:4n-6)</td>
<td>6.2±0.3*</td>
<td>7.1±0.3</td>
<td>0.035</td>
</tr>
<tr>
<td>Sum n-6 PUFA†</td>
<td>27.9±0.5</td>
<td>26.6±0.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data presented as mean±SE. † indicates that sum includes more FA than those shown. P values by Student’s T-test. * = p<0.05; NS=p>0.05.

SFA- Saturated fatty acids; MUFA- monounsaturated fatty acids; PUFA- polyunsaturated fatty acids; n-6- omega-6 PUFAs; n-3- omega-3 PUFAs; LA- linoleic acid; ARA- arachidonic acid.
5.3.5.2 Diet Analysis

Analysis of 3-day food records, provided in Table 5-12, revealed that there were no significant differences in baseline diets between groups. Over the treatment period, there were no significant changes in energy intake between or within groups. As described in Chapter 4, participants were instructed to follow a hypocaloric diet of their estimated energy requirements (EER) less 500 kcal to promote weight loss. The energy provided by the prescribed supplement amount was included in the daily energy intake permitted by the hypocaloric diet. As a result, kcal from the supplement further displaced daily kcal intake from other foods, and this was reflected in the serving sizes listed in each individual’s meal plan. For individuals in the Salba group, this meant consuming 1346.5±33.6 kcal per day, compared to mean baseline energy intake of 1751±97.7 kcal as determined from the 3-day food records gathered at week 0. Individuals in the control group were instructed to consume 1356.4 kcal per day, compared to reported baseline intakes of 1638.7±81.0 kcal. At week 12, participants in the Salba treatment group reported consuming 1665.6±88.0 kcal per day to achieve an estimated energy deficit of 181 kcal/day, compared to the oat bran group who reported energy intakes of 1746.5±130.4 kcal/day for a negative energy balance of 110 kcal/day (p=0.21). At week 24, reported mean energy intakes were 1783.0±126.3 kcal for the Salba group and 1740.1±118.4 kcal for the oat bran group, resulting in an estimated energy deficit of 64 and 116 kcal, respectively (p=0.77).

For analyses of macronutrient intakes and other nutrients of interest, see Table 5-12. Reported fibre intake increased significantly from baseline at weeks 12 and 24 in both groups. In addition, n-3 intake increased significantly from baseline in the Salba group at weeks 12 and 24 compared to the oat bran group (p=0.028). Intake of n-6 also increased significantly by week 24 in the Salba group and by weeks 12 and 24 in the oat bran group, although there was no significant
difference between groups. No significant changes were seen between or within groups for any of the other nutrients.
Table 5-12. Comparison of the nutritional composition of the diet including study supplements between each of the treatment groups at baseline, middle and end of treatment as reported by participants in 3-day food records. For all parameters n=58.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Salba (n=27)</th>
<th>Oat Bran (n=31)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
</tr>
<tr>
<td>Total energy kcal</td>
<td>1751.1±97.7</td>
<td>1665.6±88.0</td>
</tr>
<tr>
<td>Carbohydrate g</td>
<td>198.7±10.2</td>
<td>171.8±11.3</td>
</tr>
<tr>
<td>(% total kcal)</td>
<td>(46.8±1.9)</td>
<td>(45.0±1.8)</td>
</tr>
<tr>
<td>Total Fibre g</td>
<td>27.2±1.5</td>
<td>38.9±2.7</td>
</tr>
<tr>
<td>Protein g</td>
<td>78.6±5.3</td>
<td>76.8±5.0</td>
</tr>
<tr>
<td>(% total kcal)</td>
<td>(18.1±0.7)</td>
<td>(20.2±0.8)</td>
</tr>
<tr>
<td>Fat g</td>
<td>71.3±6.6</td>
<td>60.0±5.2</td>
</tr>
<tr>
<td>(% total kcal)</td>
<td>(35.1±1.7)</td>
<td>(34.8±1.6)</td>
</tr>
<tr>
<td>SFA % total kcal</td>
<td>10.3±0.9</td>
<td>9.4±1.0</td>
</tr>
<tr>
<td>MUFA % total kcal</td>
<td>9.5±0.8</td>
<td>7.2±0.7</td>
</tr>
<tr>
<td>PUFA % kcal</td>
<td>15.3±1.2</td>
<td>18.2±1.0</td>
</tr>
<tr>
<td>n-6 g</td>
<td>9.3±1.3</td>
<td>9.1±1.0</td>
</tr>
<tr>
<td>n-3 g</td>
<td>1.4±0.2</td>
<td>9.0±0.3</td>
</tr>
<tr>
<td>Calcium mg</td>
<td>609.9±46.4</td>
<td>656.0±89.5</td>
</tr>
</tbody>
</table>

Data are mean±SE. Values with different superscripts indicate significance at p<0.05 by ANOVA and Tukey-Kramer test between groups or Bonferroni test within groups. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFAs), omega-6 PUFAs (n-6), omega-3 PUFAs (n-3).
Chapter 6  
Discussion and Conclusions

6.1 An Overview of the Results

6.1.1 Effects on Weight

Our main hypothesis was that incorporating Salba into a hypocaloric diet would result in greater weight loss over 24 weeks compared to oat bran control. After 24 weeks of supplementation with 35.2±0.2g of Salba, participants’ mean weight decreased by 1.8±0.5 kg (2.3±3.3%) of their initial body weight. This was significantly more weight loss than participants in the oat bran group, who experienced a mean weight loss of 0.5±0.4 kg (0.4±3.1%) of their baseline body weight by week 24. Even by week 18, weight loss was significantly greater in the Salba group compared to oat bran control. While a significant reduction in body weight from baseline was seen within the Salba group at weeks 12, 18 and 24, no significant change in body weight occurred over the study period in the oat bran group. Clinical guidelines recommend a reduction in energy intake by 500-1,000 kcal/day in order to achieve measurable weight loss (15) and reductions of only 100-200 kcal were achieved in the current study. As such, it is not surprising that relatively small, clinically insignificant amounts of weight loss occurred over the study period.

In a previous study, Salba was observed to promote subjective satiety compared to an energy- and fibre-matched control supplement (221). It is possible that greater satiety was experienced in the Salba group resulting in lower energy intake and greater weight loss. However, satiety data was not collected in the current study, and no difference in reported energy intake was observed when comparing the Salba and oat bran groups. This discrepancy may be explained by the inherent inaccuracies present in self-reported diet records. This measurement tool is prone to bias, as are other self-reported measures of eating habits. Assessment of dietary intake is challenging, as
accuracy of the tool must be balanced with respondent burden. Compared to direct observation, both overestimation and underestimation of food intake has been reported with food records (245). A study of behavioural changes in obese men as a result of recording food intake showed that underreporting of total fat consumed often occurs (246). Food records can also be labour-intensive for participants, which may result in simplification or omission of information provided (245). Although efforts were made to clarify ambiguous entries in food records, this was not always possible. In such cases, determination of the specific type of food eaten or preparation method used depended on the discretion of the researcher.

The results of the current study contradict the findings of another weight loss study conducted by Nieman et al. using black chia seeds (223). The differences in the efficacy of Salba on weight loss in our study compared to the chia study may be due to methodological limitations of the chia study, as described in Chapter 2. Most importantly, the chia study did not include a dietary intervention. Participants were instructed to consume 50 g of chia per day in addition to their usual diet. As this amount of chia provides approximately 250 kcal, it is possible that participants were consuming additional kcal above and beyond their usual diets as a result of chia supplementation. In our study, we attempted to displace kcal in the diet with Salba by including supplement kcal in the recommended daily energy intake to promote weight loss.

The literature suggests that weight loss of 5-10% of body weight improves overall health outcomes (247). In the current study, this range was not achieved, and it can therefore be argued that neither the Salba nor the oat bran groups experienced clinically relevant weight loss. Despite this, current guidelines for the prevention and treatment of overweight and obesity state that health care providers should view any weight loss as a success, and that slow, steady weight loss is the most desirable and sustainable for patients (248). A meta-analysis of lifestyle interventions showed that sustained weight loss of ≥3.5 kg can reduce the development of T2DM by up to 58%
Individuals who manage to keep even a small amount of weight off will have reduced their risk of T2DM and CVD compared to baseline (250,251). The mean weight change for study participants was -1.2±0.9 kg at 24 weeks. In the Finnish population, body weight increases by approximately 0.5 kg/year (252), which would be expected to be even higher in a North American population. As such, the small amount of weight loss achieved by participants in this study may still be sufficient to provide health benefits.

The macronutrient percentages of the prescribed and consumed diet, as reported by participants in 3-day food records, were compliant with both CDA and National Cholesterol Education Program (NCEP) guidelines (65,253). As expected, the addition of the study supplements to the diet significantly increased n-3 intake within the Salba group and increased fibre intake within both groups. The significant increase in reported n-6 intake within both groups may have been due to messages in the CDA meal plan recommending the selection of polyunsaturated fats and reduction of sources of saturated and trans fats (see Appendix 8). Although not statistically significant, reported saturated fat intake declined within both groups over the course of the study period.

6.1.2 Effects on Glycemic Parameters

Our secondary objective was to determine the effect of Salba versus oat bran on measures of glycemic control. No long-term effects of either of the supplements on fasting glucose, fasting insulin or A1C were seen after controlling for amount of weight loss and other confounding variables. The lack of significant change in glycemic control over time may be due to the fact that study participants were already well-controlled at baseline, with mean A1C of 6.6±0.2% in the Salba group and 7.0±0.2% in the oat bran group. These values were already at the recommended treatment targets for T2DM and may not be able to show further improvements
without increases in medication. Further lowering of A1C values beyond the treatment targets may not be necessary, and tends to increase the risk of hypoglycemia (65).

Although not shown to be statistically significant, there was a 3% decrease in A1C at 12 weeks in the Salba group and a 4% reduction in the oat bran group. Although insignificant, the A1C reduction at 12 weeks is comparable to the change seen in a 12 week RCT where participants with T2DM consumed 37g/day of Salba (221), the same dosage achieved in this study. By week 24, there was a trend towards a decrease in fasting glucose of 6% and 3% from baseline in the Salba and oat bran groups, respectively (NS). Fasting insulin levels also showed a trend towards a reduction of 2-3% in both groups after 24 weeks (NS). These results suggest that Salba does not affect glycemic control when controlling for weight loss.

Food record analysis revealed a significant increase in n-3 intake in the Salba group from baseline, with no change seen in the oat bran group. As described in Chapter 2, the type of fat found in Salba is primarily n-3 PUFAs. A systematic review of 27 RCTs on the effect of fish oil supplements on glycemic control found small but non-significant increases in A1C and FBG when compared to control. The review also included 5 studies on ALA and its effect on FBG, but inconsistent findings prevented conclusions from being drawn (254). As such, any impact of n-3 from fish oil on glycemic control cannot be generalized to include ALA. In the current study, participants were advised to limit their intakes of fatty fish to <3 servings per week and were told not to take fish oil supplements during the study period. Participants were also advised not to take ALA supplements or consume ALA-rich foods such as flax or hemp seeds. Plasma EPA and DHA levels did not differ between the groups, suggesting that minimal conversion from Salba ALA and other dietary sources occurred.

A crossover study of the effects of olive oil (placebo), marine n-3s (fish oil) and plant-based n-3s on glycemic control in well-controlled individuals with T2DM showed that over 12 weeks on
each oil, no changes in glycemic control were seen (255). In a parallel study of the effect of n-3s from 13 g/day of flax oil and 32 g/day of ground flax compared to control in well-controlled individuals with T2DM, n-3 intake did not affect glycemic control over 12 weeks (256). Our study results appear to be congruent with these findings.

Intakes of EPA and DHA ≥10 g per day have been shown to adversely affect FBG levels (257). Analysis of the 3 day food records showed reported total n-3 intake (from diet and supplements) ranged from 2.14 – 11.59 g/day in the Salba group, with a mean of 8.9±0.5 g/day over the study period. Of note, only 3 participants reported total n-3 intakes ≥10 g per day during the study, and these intakes included ALA from Salba (258). As the mean reported intake of Salba was 35.2±1.2 g/day, and Salba contains 19.8 g of n-3 per 100 g, mean n-3 intake from Salba was 7.0 g/day. Based on these daily intakes, any adverse effect of n-3 on glycemic control is unlikely, which is supported by the stability of the markers of glycemic control observed in the current study.

6.1.3 Effects on Obesity-Related Outcome Measures

Waist circumference is often used as an indicator of abdominal adiposity and is a component of the metabolic syndrome (136). By week 24, waist circumference had decreased significantly from baseline in the Salba group compared to the oat bran group. Hip circumference was not significantly different between groups, although a reduction was seen within the Salba group by week 24. Taken together, these anthropometric data suggest that the majority of the weight loss that occurred in the study may have been from the abdominal region. However, these findings are not supported by %BF as measured by BIA and DXA. No significant differences in %BF were seen within or between groups using either of these methods. When interpreting the lack of change in %BF together with the significant reduction in body weight in the Salba group, it appears that fat loss did not occur and that the reduction in body weight may have been due to
reductions in lean body mass. However, this speculation is not supported by the DXA data (See Figure 5-7). Upon further examination, this discrepancy may be explained by the high variation in %BF between participants. The high standard error for these and other variables may have made any statistical differences between groups more difficult to detect.

Another potential explanation for the lack of change in %BF may be measurement errors associated with BIA and DXA. Technical error associated with BIA and DXA are relatively low at <2% and <3%, respectively (259); however, both are prone to precision error. BIA measurements are more prone to error as they are sensitive to changes in body temperature as well as differences in posture and body shape (259). In addition, water distribution between the intra and extracellular spaces have different specific resistivity and may serve as an additional source of error (259). DXA scans use a three-compartment model to assess body fat, which relies on the accurate estimation of hydration of fat free mass (260) and tissue depth (259). A recent study assessing the validity of using DXA for measuring %BF in obese men and women observed high prediction error (±4% BF) when comparing intraindividual trends over time. The authors concluded that the high degree of error appeared to be related to greater tissue thickness in obese individuals, making DXA less valuable for observing trends in body composition over time in this population (261). In the current study, participants in the Salba group had a mean reduction in %BF as measured by DXA of 4.8±3.8% compared to a mean increase in the oat bran group of 2.0±4.1% over 24 weeks (NS). It is possible that greater differences were not seen within or between groups due to the lack of validity of DXA for measuring %BF over time in obese individuals.

While total %BF did not appear to change, it is possible that fat redistribution may have occurred. Regional fat distribution measured by DXA supported the waist and hip circumference findings, showing significant reductions in android and gynoid fat within the Salba group of approximately
4% and 7%, respectively, over 24 weeks. However, no significant differences were detected between the two treatment arms.

While there was no significant change in total %BF, the significant reduction in waist circumference in the Salba group compared to the oat bran group and the significant reductions in % android and % gynoid fat observed within the Salba group may support the use of Salba as an adjunct to a hypocaloric diet for the treatment of abdominal adiposity. Reducing abdominal adiposity is particularly important in individuals with T2DM, as waist circumference is positively correlated with hypertension, dyslipidemia, and coronary heart disease in this population (262,263).

In the current study, changes in satiety-related hormones over 24 weeks may help to account for the greater weight loss seen with Salba supplementation versus oat bran. Fasting adiponectin levels increased significantly from baseline with Salba supplementation and compared to oat bran over 24 weeks. Central and peripheral administration of adiponectin has been shown to promote weight loss by reducing energy intake and increasing energy expenditure (264). As such, the increase in fasting adiponectin levels with Salba supplementation may have played a role in promoting satiety and helping this group achieve greater weight loss compared to oat bran control. However, this theory is not supported by data from the 3 day food records. While energy intake from 3 day food records did not change significantly over the study period, the significant weight loss seen in the Salba group calls the accuracy of this self-reported data into question. Energy intake or expenditure must have changed enough to permit a significant difference in body weight within the Salba group and compared to the oat bran group. Weight loss has been shown to increase adiponectin levels in individuals with T2DM and in healthy participants (265); however, the increases in adiponectin in the Salba group were still seen after controlling for changes in body weight.
Significant reductions in fasting ghrelin were seen within the Salba group over 24 weeks, independent of change in body weight and other possible confounders. Due to high standard deviation, the change in fasting ghrelin levels was not significantly different between groups or within the oat bran group. Ghrelin is the only gut hormone known to increase food intake (19) and fasting levels are higher in obese versus lean individuals (266). Weight loss has been shown to increase ghrelin levels, leading to greater hunger and therefore making it difficult to sustain an energy-reduced diet (23). The significant reduction in fasting ghrelin levels that occurred within the Salba group may provide a partial explanation for the greater weight loss seen in the Salba group versus the oat bran group, although the energy deficit achieved was not statistically different between the groups.

No significant changes were seen in fasting PYY for either of the treatment groups. This may be because PYY appears to have a greater impact on appetite post-prandially rather than in the fasted state (27). In both lean and obese individuals, administration of exogenous PYY has been shown to promote satiety and reduce energy intake (267,268). Weight loss has been shown to increase fasting PYY levels which may help to promote maintenance of weight loss (37). In the current study, after controlling for weight change over time, change in fasting PYY did not differ between or within groups.

The significant increase in the anorectic hormone adiponectin with Salba supplementation compared to oat bran, along with trends towards reduced fasting ghrelin levels in the Salba group suggests that gut hormones may provide an explanation for the mild weight-reducing effect of Salba. These results are difficult to interpret as only a few satiety-related hormones were analyzed in the current study.
6.1.4 Effects on Cardiovascular Disease Risk Factors

After controlling for amount of weight change from baseline and other confounding factors, dietary supplementation with Salba over 24 weeks did not significantly affect blood pressure and lipid levels compared to oat bran control or within either of the groups. However, CRP levels were significantly reduced in the Salba group compared to the oat bran group by week 24.

The lack of change in BP over the study period may be due to the fact that optimal BP control had already been achieved at baseline, with 19 participants in the Salba group and 15 in the oat bran group on antihypertensive medication. Furthermore, the current literature suggests that a weight loss of at least 3 kg is needed in order to improve blood pressure (50), and this was not achieved by either of the treatment groups. In contrast to our findings, a randomized crossover study of the effects of ALA on CVD risk factors in hypercholesterolemic participants showed a significant reduction in DBP with the ALA-rich diet (269). The effect of ALA on BP is hypothesized to occur as a result of conversion of ALA into EPA, which modifies the eicosanoid pathway and decreases the production of vasoconstrictive prostaglandins (270). However, serum lipid analysis did not show a significant difference in EPA levels between the Salba and oat bran groups at the end of our study, suggesting that sufficient conversion did not occur. A crossover study of the impact of Salba supplementation in individuals with T2DM showed a reduction in DBP of 3 mm Hg over 12 weeks, although this was not significant (221). SBP was significantly reduced by 9±4 mm Hg during Salba treatment compared to an increase of 10±1 mm Hg on wheat bran control. However, the authors mention that baseline BP was not equivalent between treatment phases, suggesting that the 4-week washout period between treatments may not have been sufficient (221).

In this study, no significant changes in serum total cholesterol, high-density lipoprotein, low-density lipoprotein or triglycerides were observed in either treatment group. It was hypothesized
that the oat bran group would experience significant reductions in LDL levels compared to the Salba group. Oat fibre has been shown to effectively lower LDL by 5-10% as part of a diet low in saturated fat in individuals with hyperlipidemia and T2DM (271,272). In the current study, LDL levels did not change with oat bran supplementation over time or compared to the Salba group. Health Canada and the FDA maintain that 3 g/day of oat β-glucan, the amount provided by approximately 40 g of oat bran, is sufficient to reduce LDL (227,273,274). A meta-analysis on the efficacy of oats in improving hyperlipidemia demonstrated that 2-10 g/day of β-glucan can lower total cholesterol by 0.08 - 0.40 mmol/L and LDL by 0.075 - 0.37 mmol/L (271). In the current study, the amount of β-glucan in the oat bran-based supplement was not measured. As such, it is not possible to accurately determine the daily intakes of β-glucan from the oat bran supplement in order to compare them to the amounts required for health benefits. It is possible that the amounts of β-glucan consumed in the current study were insufficient to permit significant changes in LDL levels within the oat bran group and in comparison to the Salba group.

A Cochrane review of 23 RCTs on the effect of marine-derived n-3s on CVD risk factors in individuals with T2DM showed that n-3 supplementation reduces serum triglycerides levels in this population (275). In the current study, 12 weeks of Salba supplementation showed a trend in the reduction of TAGs by approximately 13% from baseline, although this was not significantly different from baseline or compared to the oat bran group. The lack of significant change in TAG levels despite high n-3 intakes with Salba supplementation may be due to mechanistic differences between marine- and plant-derived n-3s. Epidemiological evidence supports the association between ALA intake and reduced risk of CAD and MIs (276,277). However, the lack of RCTs on the subject prevents any conclusions regarding causality from being drawn. At this time, the impact of ALA supplementation on TAG levels in individuals with T2DM cannot be determined (278).
The literature suggests that increases in dietary n-3 may raise LDL levels, although results are conflicting (275). A crossover study of the effects of 12 weeks of supplementation with each of ALA, fish oil and olive oil on serum lipids in individuals with T2DM showed that no differences in lipid levels occurred on any of the treatments (255). In the Lyon Diet Heart Study, adding n-3s to a low-fat, high-carbohydrate Mediterranean diet did not impact TAGs, LDL, HDL, or TC but was associated with a 65% reduction in coronary heart disease (CHD)-related mortality (279). In the current study, while Salba supplementation significantly increased n-3 intake from baseline, similar to the Lyon study, the addition of n-3 did not appear to affect serum lipids levels.

The reduction in CRP in the current study supports the anti-inflammatory effects of ALA-rich foods such as Salba. Inflammation plays an important role in T2DM, obesity and CVD, and so reductions in inflammatory markers such as CRP may be useful as measures of overall health risk (280). However, prospective studies evaluating the effects of dietary supplementation with marine and plant-based n-3s have not demonstrated significant changes in CRP levels (281-283). In an 8 week study, participants with abdominal adiposity were randomized to an ALA-enriched diet via supplementation with flaxseed oil (5% of energy from ALA) or control (usual diet) and inflammatory markers were measured at baseline and the end of the study (284). No significant changes in inflammatory markers were seen, but a possible limitation of the study was the participants were free of chronic disease and had normal levels of inflammatory markers at baseline. Therefore, there may have been little room for further reduction in inflammation over the study period (284). Despite these contradictions, the improvement in CRP in the current study supports the findings of a previous long-term study where CRP decreased by 7.0 ±2.3% after supplementation with 37 g/day of Salba over 12 weeks (221). This suggests that the anti-inflammatory effects of Salba supplementation may not be related to the n-3 content as previously thought, but may be explained by other nutrients.
Analysis of the 3 day food records showed an increase in n-6 intake in both the Salba and oat bran groups from baseline. Plasma phospholipid fatty acids at week 24 showed that while total n-6 levels did not differ between groups, linoleic acid (LA) levels were significantly higher in the Salba group and arachidonic acid (ARA) levels were higher in the oat bran group. LA is the shortest of the n-6s and is the primary n-6 consumed in the human diet. A recent systematic review of 15 RCTs showed that LA intake does not lead to increases in a variety of inflammatory markers, including CRP (285). Very little LA is converted in vivo into ARA, with most ARA coming from dietary sources (286). ARA has been shown to exhibit inflammatory effects by triggering the production of proinflammatory eicosanoids and by blocking production of anti-inflammatory eicosanoids by EPA and DHA (285). The higher ARA content in the plasma phospholipids of the oat bran group may explain the higher CRP values seen at week 24 compared to the Salba group. In addition, adiponectin has been shown to have anti-inflammatory effects (27) and showed a significant increase in the Salba group over 24 weeks. It is also possible that the high antioxidant capacity of Salba influences markers of inflammation such as CRP. More research is needed to determine which components of Salba may be responsible for its possible anti-inflammatory effects.

6.2 Study Limitations

There are several limitations to the current study, which must be considered when interpreting these preliminary results.

Power analysis revealed that in order to observe a significant effect on the primary outcome measure, body weight, 132 participants needed to be recruited for this study. This power calculation was based on achieving a clinically significant body weight reduction of 5% of initial body weight. In the current study, a statistically significant effect of treatment on weight change over time was seen after analyzing data from only 58 participants. It is possible that differences
in weight change between the groups may have reached significance at earlier time points with
greater numbers of participants. In addition, power analyses were not calculated for other
outcome variables used in this study, so a larger sample size may have been needed to detect
differences between the treatment groups. In addition, participants who dropped out of the study
were not followed afterwards, so it is possible that those who dropped out were not as successful
in achieving weight loss. Exclusion of these participants is a limitation of the current study.

Participants in this study had stable, well-controlled T2DM (A1C 6.9±0.2% [mean±SE]) and
blood pressure (SBP 122.9±2.3 mm Hg; DBP 71.7±1.4 mm Hg). Many of the participants were
already taking antihyperglycemic agents (n=52) and/or antihypertensive agents (n=34), which
were kept unchanged throughout the treatment period of the study. The lack of significant
changes in markers of glycemic control observed in this study may reflect that optimal baseline
glycemic control was achieved by the participants’ underlying pharmacological therapy. Further
improvements in glycemic control may not have been possible in this group after controlling for
the effects attributable to weight loss. Furthermore, the diets of the study participants may have
already been reasonably healthy at baseline, showing less room for improvement than the typical
overweight or obese individual with T2DM. Because participants were recruited using
advertisements, it is likely that individuals already interested in nutrition and healthy eating self-
selected for participation in the current study. While recent statistics indicate that the average
North American consumes less than 15 g of dietary fibre per day (73), participants in this study
reported consuming 26.5±1.9 g of fibre/day at baseline, within the recommended intake of 21-38
g/day for the general population (287) and recommendations of 25-50 g/day for individuals with
T2DM (65). This provides further support for the possibility that study participants may have had
healthier baseline diets than the average overweight or obese individual with T2DM due to self-
selection bias.
Maintaining compliance with protocols in dietary and supplement-based studies is a common challenge, particularly over the long-term (288). Supplement compliance was not significantly different in either treatment group or over time, but reliance on self-reporting of supplement intake brings the accuracy of these findings into question. Plasma fatty acids were measured at the final study visit to compare differences in ALA and other n-3s between the study groups. While a significantly higher level of ALA in the plasma of the Salba group indicated that they were consuming more ALA than the oat bran group, no information on oat bran compliance can be gathered from this data. As indicated in Table 4-3, ALA samples were collected at baseline and at the end of the study, but not during the study period. In addition, baseline samples were not analyzed for this thesis due to time and budgetary constraints. As such, conclusions regarding the change in plasma ALA, EPA and DHA over time cannot be drawn. As a result of these limitations, information on compliance cannot be reported with any certainty.

While every effort was made to ensure the study supplements were similar in appearance, it is possible that study participants were able to identify which treatment group they were assigned to. Because oat bran is readily available and was likely familiar to the study participants, the oat bran-based control supplement may have been recognizable. Salba may not be as familiar, but is becoming increasingly available in many grocery and health food stores. A previous long-term study suggests the taste of *Salvia hispanica* is relatively neutral, as only 40% of individuals randomized to chia seeds were able to positively identify they were taking the seeds and not the placebo (223). In the current study, supplements were ground in order to ensure a similar appearance between the Salba and oat bran supplement. However, this may have increased the odour of the Salba, making it easier to identify. All participants were encouraged to store their supplements in a refrigerator in order to minimize possible odours from the Salba. Despite these attempts to make the supplements indistinguishable from each other, it is possible that some of the participants were able to identify which treatment they were on. Any unblinding of
participants during the study period may have impacted supplement compliance, although participant-reported compliance did not differ between groups. As previously discussed, compliance cannot be determined with much certainty in the current study due to methodological limitations. Participants were not directly asked whether they could identify their study supplements, but this should be considered for future studies.

The potential impact of physical activity on body weight was not controlled for in the current study. At baseline, all participants were classified as either sedentary or lightly active based on estimated time spent sitting, standing, walking and exercising per day collected from self-reported physical activity records and listed occupation. Each participant remained in the same physical activity category throughout the study. While participants were encouraged to maintain their baseline levels of physical activity throughout the study, it is possible that there were some minor variations over time or between the groups that may have impacted on the outcome variables.

Participants were given pedometers to track their total number of steps per day, but uptake was poor. The majority of participants either lost their pedometers or found them temperamental, which led to limited use of them during the study. Only a few participants reported any pedometer results, prohibiting analysis of this data.

Another source of methodological error could have arisen from measurements of waist and hip circumference. Every effort was made to ensure that the same individual took anthropometric measurements for each participant to maximize consistency. Despite this, waist and hip circumferences have been shown to have relatively high measurement error and ethnic bias (289). Waist circumference measurements are especially prone to variation in males with higher BMIs as the waist can be challenging to locate in these individuals (289).
Another methodological limitation of the current study was the analysis of only three satiety-related hormones, and in the fasted state exclusively. This was primarily due to factors affecting feasibility, such as cost and increased participant burden caused by taking additional blood samples post-prandially. GLP-1 is another gut hormone that affects satiety and is of particular interest in T2DM, as described in Chapter 2. Similarly to PYY levels, GLP-1 levels also increase as a result of soluble fibre intake, which may also suppress secretion of ghrelin (290). When creating the protocol for the current study, GLP-1 was not prioritized for analysis as the GLP-1 response appears to be related to nutrient ingestion, with only secondary effects on satiety (192). PYY and ghrelin were selected for analysis in the current study as irregularities in their expression and secretion are associated with chronic diseases related to lifestyle causes, namely obesity. Adiponectin was selected as it increases insulin sensitivity and plays an important role in glucose and lipid metabolism, in addition to its anorexigenic effects (21).

Hormones that were considered but not selected for analysis, such as GLP-1, pancreatic polypeptide, cholecystokinin and oxyntomodulin, appear to regulate only short-term energy intake via signaling for meal termination (291,292). As such, measuring these hormones in the fasted state but not post-prandially would be unlikely to provide useful data. Furthermore, GLP-1 has a rapid onset and a short half-life of only 1-2 minutes (292), and cholecystokinin has a plasma half-life of several minutes (293). Pancreatic polypeptide has also been shown to be rapidly degraded once it enters circulation (292), rendering these gut hormones difficult to measure accurately.

Despite the rationale for selecting only a few gut hormones, satiety is impacted by a complex interplay between multiple hormones. As such, any interpretation of the gut hormone changes in the current study must be interpreted with caution, as other satiety-regulating hormones were not measured.
6.3 Future Directions

The results presented here provide rationale for the continuation of this study to include a weight maintenance period. The consumption of 35.2±0.2 g/day of Salba or 40.0±0.3 g/day of the oat bran blend appears to be safe, as no adverse events were reported. Weight loss and reduction in waist circumference were significantly higher in the Salba versus the oat bran group after 24 weeks, yet conclusions regarding whether this weight loss can be maintained over time cannot be drawn. It was originally intended that a 24-week weight maintenance phase would follow the 24-week weight loss phase, and would be included in the study results. However, due to time limits to complete graduate studies, insufficient numbers were recruited into the weight maintenance phase, preventing analysis of this data.

Despite these challenges, guidelines for the treatment of overweight and obesity published by the NIH advise that after patients spend 6 months on a weight reduction diet, a weight maintenance phase should immediately follow. After a period of successful weight maintenance, further weight loss can be attempted if needed; however, the minimum goal is to prevent further weight gain (294). According to the NIH Evidence Report, a weight loss intervention must last a minimum of 12 weeks and follow-up a minimum of 16 weeks (294). As such, following a weight loss study with a period of weight maintenance is advisable.

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 an RCT on meeting lifestyle goals which included >4 hrs/day of moderate physical activity, <30% total energy intake from fat, and 15g of fibre/1000kcal daily, participants with the highest success rates were those who already met the objectives at baseline (295).
In the current study, no specific functional component of Salba can be implicated in the potential health benefits observed, as the study supplements were matched for energy and fibre only. This necessitates the follow up of the current study with additional mechanistic studies to determine the mode of action by which Salba may help promote weight loss. Future studies may attempt to match the content of other dietary components, such as protein or n-3, in order to determine the specific nutrients that may be responsible for the potential effects of Salba on health.

6.4 Conclusions

The hypothesis that supplementation with Salba as part of a hypocaloric diet assists with weight loss in overweight and obese individuals with T2DM is supported by the findings presented here. While waist circumference was significantly reduced in the Salba group, total %BF and % android fat measured by DXA were not significantly different between the groups. Results of the current study do not support the hypothesis that Salba has a positive effect on glycemic control compared to the oat bran-based control supplement. The hypothesis that Salba supplementation would improve CVD risk factors was only true for CRP; all other CVD risk factors did not significantly differ within or between groups. It was hypothesized that LDL levels would be significantly reduced in the oat bran control group compared to the Salba group, but this hypothesis was not supported by this study. Several limitations exist in the presented research. For instance, not achieving the sample size calculated by power analysis may have prevented weight-related and other measures from reaching statistical significance. It was hypothesized that Salba supplementation would not affect safety parameters or cause adverse side effects, which was supported by the study findings and is consistent with the previous literature on Salba. Given the increasing prevalence and rising healthcare costs attributable to T2DM and obesity, further investigation into Salba as a potential supplement to hypocaloric weight loss diets is needed. In particular, investigation into whether Salba can assist in long-term maintenance of weight loss is an area for further research.
References


(25) Purnell JQ, Weigle DS, Breen P, Cummings DE. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. Journal of Clinical Endocrinology & Metabolism 2003;88(12):5747-52.


(82) Riddle M. Combining sulfonylureas and other oral agents. The American journal of medicine 2000;108:15S.


(91) Izadpanah A. A Short-term Diet and Exercise Intervention Ameliorates Inflammation and Markers of Metabolic Health in Overweight/Obese Children. The Journal of Endocrinology and Metabolism [epub ahead of print], 1-19. 6-19-2012.


(93) Saltevo J, Laakso M, Jokelainen J, Keinänen-Kiukaanniemi S, Kumpusalo E, Vanhala M. Levels of adiponectin, C-reactive protein and interleukin-1 receptor antagonist are


(97) Greenfield JR, Campbell LV. Relationship between inflammation, insulin resistance and type 2 diabetes: 'cause or effect'? Curr Diabetes Rev 2006 May;2(2):195-211.


(172) Wu JHY. Omega-3 Fatty Acids and incident Type 2 Diabetes A Systematic Review and Meta-Analysis. 2012.


(186) Schaaßma G. The protein digestibility corrected amino acid score. The Journal of nutrition 2000;130(7):1865S-7S.


(200) Teegarden D. Calcium intake and reduction in weight or fat mass. The Journal of nutrition 2003;133(1):249S-51S.


(220) Vertommen J. Efficacy and Safety of 1 Month Supplementation of SALBA (Salvia Hispanica Alba) Grain to Diet of Normal Adults on Body Parameters, Blood Pressure, Serum Lipids, Minerals Status and Haematological Parameters. Results of a Pilot Study. 2005.


(225) Choleva L. The Effect of Salvia hispanica L.(Salba) on Weight Loss in Overweight and Obese Individuals with Type 2 Diabetes Mellitus. 2011.


(234) EMD Millipore. Assay Procedure for Human Adiponectin ELISA kit (Cat. # EZHADP-61K) . 5-25-2012. St. Charles, Missouri, EMD Millipore.

(235) EMD Millipore. Assay Procedure for Human PYY (Total) ELISA Kit 96-Well Plate (Cat. # EZHPYYT66K). 2-12-2010. St. Charles, Missouri, EMD Millipore.


Appendix 1: 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/h^2) __________ (must be 25-40 Kg/m^2)

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 Control in Overweight and Obese Individuals with Type 2 Diabetes

PRINCIPAL INVESTIGATOR
Dr. Alexandra Jenkins, PhD, RD
Post-Doctoral Fellow
Risk Factor Modification Centre
St. Michael’s
Tel: (416) 864-6060 ext 2598
E-mail: alexandra.jenkins@utoronto.ca

QUALIFIED INVESTIGATOR
Dr. Amir Hanna, MD, FRCPC (C)
Endocrinologist, Division of Endocrinology and Metabolism,
St Michael’s
Professor Emeritus, Faculty of Medicine, University of Toronto
Tel: (416) 867-3721
Email: HannaA@smh.ca

CO-INVESTIGATORS
Dr. Vladimir Vuksan, PhD
Professor, Departments of Medicine and Nutritional Sciences
Faculty of Medicine, University of Toronto
Associate Director, Risk Factor Modification Centre
St. Michael’s
Tel: (416) 864-5525
Email: v.vuksan@utoronto.ca

Dr. Arya Sharma
Canadian Obesity Network
University of Alberta
Edmonton, AB

STUDY SPONSOR: Canadian Diabetes Association

STUDY COORDINATOR
Christy Brissette, RD, MSc Candidate
Department of Nutritional Sciences
Faculty of Medicine, University of Toronto
Tel: (416) 864-6060 ext. 2596
Email: BrissetteC@smh.ca
Before agreeing to participate in this research study, it is important that you read and understand this research consent form. This form provides all the information we think you will need to know in order to decide whether you wish to participate in the study. If you have any questions after you read through this form, ask your study doctor or study personnel. You should not sign this form until you are sure you understand everything on this form. You may also wish to discuss your participation in this study with your family doctor, a family member or close friend. It is important that you are completely truthful with your study doctor with respect to your health history and any medications you may be taking in order to prevent any unnecessary harms to you should you decide to participate in this study.

PURPOSE OF THE RESEARCH

The primary objective of this clinical trial is to evaluate whether adding Salba, a high fibre seed, to a calorie-reduced diet will result in greater weight loss over 24 weeks in overweight and obese individuals with type 2 diabetes when compared to an oat bran control.

Salba is a single variety of a seed botanically known as Salvia hispanica L. that was once used as a food and remedy by the ancient Aztecs, who referred to it as ‘Running Food’ because messengers claimed that they could run all day long on just a handful of these seeds. Salba has a unique nutrient composition which includes dietary fibre, protein, omega 3 fatty acids, and numerous vitamins and minerals such as calcium, vitamin C, iron, and potassium, which can possibly assist in weight loss and control of blood sugar levels.

The intake of dietary fibre is linked to improved health and decreased risk of obesity, diabetes and heart and blood vessel disease. Based on a previous study done by our research group at the Risk Factor Modification Centre, it was found that Salba improved blood sugar and had beneficial effects on blood pressure control. As a high fibre food, Salba may also possibly assist with body weight management. However, the benefits of Salba intake have not yet been tested in patients with type 2 diabetes.

This study will try to determine whether Salba can help weight loss and improve blood sugar control and other markers of health, including clotting factors and markers of inflammation, in patients with type 2 diabetes.

DESCRIPTION OF THE RESEARCH

If you consent to participate in this study, you will be given a personalized diet and a supply of either Salba or oat bran sprinkles. The study is double-blind, which means that neither you nor the investigators know whether you are taking Salba or the oat bran placebo. A placebo is an inactive substance that looks identical to the study treatment but has no effect on your body. Placebos are used to compare the effectiveness of a treatment against the effect of no treatment. There will be two distinct study groups, one which will receive Salba supplements and the other which will receive oat bran placebo supplements. Whether you are assigned to consume Salba or placebo is random; randomization means that you are assigned by chance, like flipping a coin, to the Salba treatment group or to the placebo group and ensures fairness in the study. Randomization will occur prior to the start of the treatment period and will ensure that each treatment group has similar characteristics based on body weight, waist
circumference and gender. This process will not be carried out by the study investigators, coordinators, or anyone else related to the study, in order to ensure proper blinding. The study investigators will provide you with either the placebo or Salba supplement (free of charge) and give you recommendations on how to incorporate the supplement into your diet. The total duration of the study will be approximately 24 weeks, during which time you will follow a diet designed by a registered dietitian.

PROCEDURES

Screening Visit

If you agree to participate in this study and have signed the consent form, you will be asked to come to the Risk Factor Modification Centre at St. Michael’s Hospital, 30 Bond Street, Queen Street entrance, 10th floor, for a screening visit, which will last about 30 minutes. You will have your weight and blood pressure measured. If you meet the criteria and are willing to join the study, you will be invited to return to the clinic at the Risk Factor Modification Centre for your initial study appointment. You will receive a 3-day food record form to be completed by the next study visit. You will also be asked to complete and return a detailed questionnaire concerning your medical history, drugs and medication use (including, herbs, vitamins and minerals), alcohol intake, exercise pattern, and diet.

Initial Study Appointment

You will have to fast overnight (nothing to eat or drink) for 10-12 hours before your first study visit. This is needed because some blood test results may be affected by food.

During the screening visit, the following procedures will be performed:

a) Physical exam, including measurement of blood pressure, height, weight, waist to hip ratio (measured with a measuring tape)

b) Body fat percentage and body composition, measured by DEXA. Dual Energy X-Ray Absorptiometry (DEXA) uses X-ray equipment to measure your total body composition while you lie down on a padded table. This procedure lasts 10-30 minutes.

c) Blood samples drawn to test fasting blood sugar, HbA1c (a blood test which tells your doctor how well your diabetes is controlled), fasting insulin, C-reactive protein (hs-CRP, a marker of low-grade inflammation), adiponectin, ghrelin and PYY (hormones related to hunger and appetite), von Willebrand Factor (a marker of blood clotting), safety factors, and alpha-linolenic fatty acid (ALA, also related to hunger and appetite)

Furthermore, during this time you will also be meeting individually with a registered dietitian, who will assist you by performing the following tasks:

a) Use your 3-day food record to determine your baseline energy intake

b) Provide you with dietary advice for reducing your energy intake by 25% per day

c) Encourage you to avoid excessive consumption of high-fat foods, reduce portion sizes, and increase your daily intake of fruits and vegetables

d) Teach you to follow the Canadian Diabetes Diet Guidelines.

e) Instruct you to do at least 20 minutes of regular physical activity per day without changing other lifestyle or social habits you have
You will be randomly assigned to one of two treatment groups: Salba treatment or placebo treatment. You will receive instructions on how to incorporate the supplement into your diet and your supplement dosage, as well as a supply of your supplement to last until your next study visit.

After the initial study appointment, you will be asked to come in for 5 more visits over 6 months: Week 2, Week 6, Week 12, Week 18, and Week 24. You will follow your weight loss diet and will be given enough supplement or placebo to last until your next visit. The schedule of your clinic visits, the procedures that are done at each visit, and the level of treatment and care you receive will be the same as all other participants.

**Study Measurements**

At every visit, the following measurements will be taken: blood pressure; height; weight; you will be asked to fill out and return a symptoms diary and physical activity record prior to the visit; you will be asked to fill out and return a 3-day diet and hunger score record for the 3 days prior to the visit. At each visit, the following additional measurements will be taken: body fat percentage and body composition analysis by the TANITA Body Composition Analyzer (this involves standing barefoot on a scale).

At Week 0 and 24, the following additional measurements will be taken: body fat percentage and body composition (measured by DEXA scan) and blood sample for the same tests as at the screening visit, plus gut peptide hormones and at Week 24, ALA.

At Week 12, the following additional measurements will be taken: blood sample for the same tests as at the screening visit.

At every clinic visit, you will be given packages of the supplement or placebo to last until your next appointment. At each visit, you have to bring all your empty and opened packages you were given at the previous visit and a new supply of the supplement or placebo will be given to you. The amount you receive may differ depending on the number of weeks until your next visit. You will always be given more than enough supplement to last until your next appointment. If you misplace or are running low on your study materials, contact the Study Coordinator as soon as possible.

You will also be advised to monitor your blood glucose daily using your home glucose monitors and record your morning fasting glucose levels every other day. You will be required to bring in your results at every visit. You will be instructed on the signs and symptoms of low blood sugar, and on what you should do in case you experience low blood sugar. If you experience fasting blood sugar level below 4mmol/L, contact the investigator immediately. It is your duty to inform your health care team and the study staff about any new medications not related to the main diabetes and antihypertensive treatments.

At the end of the study, after all of the data had been collected, you will be able to request unblinding, which means that study personnel can inform you as to whether you were in the Salba or oat-bran placebo treatment group.
POTENTIAL HARMs (INJURY, DISCOMFORT, OR INCONVENIENCE)
The risks of blood sampling may include dizziness, discomfort, redness, swelling, bruising,
and very rarely, infection. Side effects from taking Salba or oat bran may include bloating,
diarrhea, flatulence and abdominal cramps.

A DEXA scan involves being exposed to some X-ray radiation. The radiation dose being
used is generally thought to be safe for adults. There is a slight chance of cancer from
excessive exposure to radiation. Pregnant women are advised not to have a DEXA scan,
therefore it is important for you to tell your study doctor if there is a possibility you are
pregnant.

It is important that you are completely truthful with your study doctor with respect to your
health history and any medications (including any natural health products) you may be
taking, and that you follow the instructions of your study doctor in order to prevent any
unnecessary harms to you should you decide to participate in this study.

In the case of an adverse event, which in the opinion of the study doctor, Dr. Hanna, may be
related to the study, unblinding will occur.

REPRODUCTIVE RISKS
Pregnancy and this study are not compatible. Due to the risk or potential risk to the unborn
child, women who are pregnant or planning to become pregnant are excluded from this study.
Only women who are considered non-pregnant or post-menopausal are permitted to enroll in
this study. Post-menopausal includes those females with more than a year of no menstrual
periods. Unless you have had a hysterectomy, a tubal ligation, are post-menopausal, or not at
risk of pregnancy, you are advised to practice and discuss appropriate family planning with
your doctor. If you are within childbearing age but are not pregnant and have no plans of
becoming pregnant in the near future you will be asked to take a pregnancy test prior to being
enrolled in the study. Only those who are confirmed non-pregnant by a pregnancy test will
be able to participate in this study. If you become pregnant once commencing the study you
must stop taking the study product and inform the study investigators.

POTENTIAL BENEFITS
There may be no direct benefit to you for participating in this study. However, better glucose
control, better weight management, and better blood pressure control may be achieved with
participation in the study for the duration of the trial. Others may benefit from the
knowledge gained through your participation.

COMPENSATION AND REIMBURSEMENT
Compensation for the study will be transportation costs per study visit and will be paid at
each study visit. If you withdraw from the study early or if the study is terminated early, you
will be reimbursed for transportation costs for each visit for the portion of the study you did
complete.

PUBLICATION OF RESULTS
The results of this study may be presented at scientific conferences, seminars, or other public forums and they may be published in a scientific journal. You as a participant will not be identified.

CONFIDENTIALITY AND PRIVACY
The study investigators, sponsor CDA, Health Canada, coordinators, nurses and delegates (hereby referred to as “study personnel”) are committed to respecting your privacy. No other persons will have access to your personal health information or identifying information without your consent, unless required by law. Any medical records, documentation, laboratory samples or information related to you will be coded by study numbers to ensure that persons outside of the study (i.e., sponsors) will not be able to identify you, these will be kept for 25 years from the end of the study. No identifying information about you will be allowed off site. All information that identifies you will be kept confidential and stored and locked in a secure place that only the study personnel will have access to. In addition, electronic files will be stored on a secure hospital or institutional network and will be password protected. It is important to understand that despite these protections being in place, experience in similar studies indicates that there is the risk of unintentional release of information. The principal investigator will protect your records and keep all the information in your study file confidential to the greatest extent possible. The chance that this information will accidentally be given to someone else is small.

By signing this form, you are authorizing access to your medical records by the study personnel, authorized representatives of the sponsoring company: the Canadian Diabetes Association, and the St. Michael’s Hospital Research Ethics Board. Such access will be used only for purposes of verifying the authenticity of the information collected for the study, without violating your confidentiality, to the extent permitted by applicable laws and regulations.

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

PARTICIPATION AND WITHDRAWAL
Participation in the study is voluntary. You may decide not to participate or to withdraw from the study at any time without penalty and without affecting any future medical care. Due to safety concerns, you will be asked to withdraw from the study in the event of pregnancy. Participation in the study may be terminated at any time by the study doctor or the sponsor without your consent: 1) if it is judged to be in the best interests of your health; 2) if you do not meet the study requirements; 3) if the study is cancelled.
If you withdraw your consent or are terminated early for any reason:
1. No new information will be collected about you.
2. All study related information collected about you before you withdraw from the study will be kept and used for study analysis
3. You will return all study medication

NEW FINDINGS OR INFORMATION
We may learn new things during the study that you need to know, we may learn things that may make you want to stop participating in the study, if so, we will let you know of new information in a timely manner. You may also be asked to sign a new consent form discussing these new findings if you decide to continue in the research study.

COMPENSATION FOR INJURY
If you suffer physical injuries as a direct result of the study supplements, a device or procedure, you can get medical care in the same way as you would usually get any other medical treatment. Signing this form does not waive your legal rights and does not stop the investigator, sponsors, or participating institutions from their legal and professional responsibility.

WHO TO CONTACT
As a research subject, you have the right to information about the study. You may ask questions about this study at any time. You will also be informed of any significant new findings that may affect your safety or decision to remain in the study. If you have any questions or have research related concerns, please contact Dr. Alexandra Jenkins at (416) 826-3598 or Dr. Vladimir Vuksan at (416) 864-5525 Monday to Friday 9am-6pm.

RESEARCH ETHICS BOARD CONTACT INFORMATION
If you have any questions as a research subject, then you may contact the Chair of the St. Michael’s Hospital Research Ethics Board at (416) 864-6060 ext. 2557.
STATEMENT OF CONSENT

TITLE OF RESEARCH STUDY
*Efficacy and Safety of Whole Grain Salba (Salvia Hispanica L.) on Weight Control 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 form their legal and professional duties.

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

*Please check the appropriate box and initial to indicate your decision:*
(1) □ I agree that my family doctor may be notified in writing of my study participation and my study results. ________ (initial)
□ I do not agree that my family doctor may be notified of my study participation and my study results. ________ (initial)

(2) □ I wish to be notified in writing of my study results. ________ (initial)
□ I do not wish to be notified in writing of my study results. ________ (initial)

Participant name (printed): ____________________________
Participant signature: ____________________________ Date: ______________

Name & Position of Person Conducting Consent Discussion (printed): ____________________________
Signature of Person Conducting Discussion: ____________________________
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: ______________________________________

Ofﬁce use only:

Ht (cm):   Wt (kg):   BMI:

Waist: Hip Ratio:

Waist Circumference (cm):

Blood pressure (mmHg):

% Body Fat:

<table>
<thead>
<tr>
<th></th>
<th>High blood sugar</th>
<th>High blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>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.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>□ Yes   □ No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>When: ________</td>
<td></td>
<td></td>
</tr>
<tr>
<td>How high:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting glucose: ______ mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-meal glucose: ______ mmol/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c (glycosolated haemoglobin) %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sBP/dBP: ______ / ______ mmHg</td>
<td></td>
<td></td>
</tr>
</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

<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>
</tr>
<tr>
<td>Malabsorption syndrome</td>
<td></td>
<td></td>
<td></td>
<td>Active (please indicate treatment)</td>
</tr>
<tr>
<td>Crohn’s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach (gastric) ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenal ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal parasites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea (&gt; 2 liquid stools/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONDITION</td>
<td>No</td>
<td>Yes</td>
<td>Onset date</td>
<td>Present status</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----</td>
<td>-----</td>
<td>------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Constipation (≥ 3 days duration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia or Bulimia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart attack</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arrhythmia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uncontrolled hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic BP ≥ 140</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic BP ≥ 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood clotting disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychiatric conditions (i.e Depression)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONDITION</td>
<td>No</td>
<td>Yes</td>
<td>Onset date</td>
<td>Present status</td>
</tr>
<tr>
<td>Infectious hepatitis (B, C, D)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recently diagnosed infectious hepatitis A, E</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV/ AIDS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Do you experience any of the following:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained weight gain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry skin and hair</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depressed mood</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold intolerance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased cholesterol?</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nervousness/irritability</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat intolerance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased sweating</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained weight loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insomnia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pancreatic disease</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Diabetes</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any food allergies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allergies wheat bran powder or maltodextrin</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Any food intolerance</th>
</tr>
</thead>
</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 watchers, etc.)
________________________________________________________________________
________________________________________________________________________
_____
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>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belching</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flatulence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excessive urination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insomnia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disorientation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor wound healing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excessive bleeding after cuts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impaired vision</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart flutters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joint pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numbness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</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, Oatbran  ☐ Carrots, Peas, Sweet Potatoes  ☐ Barley
  - ☐ Chickpeas  ☐ Apples, Pears, Berries, Citrus fruit  ☐ Flax
- Lentils, dried Peas, Beans
- 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?
  - 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? □ 3 or more □ Two □ One
  When: ________________________________

- 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

Salvia hispanica
LOSS Trial
Subject #: 

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

<table>
<thead>
<tr>
<th>1- Much more</th>
<th>2- More</th>
<th>3-The Same</th>
<th>4- Less</th>
<th>5- Much Less</th>
</tr>
</thead>
<tbody>
<tr>
<td>15. In comparison with others my own age I think my physical activity during leisure time is…</td>
<td>[1] [2] [3] [4] [5]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

16. How many minutes do you walk and/or cycle per day to and from work, school and shopping/errands?  
Appendix 6: St. Michael’s Hospital Research Ethics Board Approval

Research Ethics Office
Telephones: (416) 864-5000 Ext. 2557
Facsimile: (416) 864-5043
E-mail: researchethics@mcmaster.on.ca

February 20, 2013

Dr. Alexandra Jenkins,
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

<table>
<thead>
<tr>
<th>REB APPROVAL:</th>
<th>Original Approval Date</th>
<th>Annual/Interval Review Date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>February 19, 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>February 19, 2014</td>
</tr>
</tbody>
</table>

Thank you for your communications dated February 10, 2013 requesting an annual review and approval regarding the above named study.

This letter will serve as an extension of the St. Michael’s Hospital (SMH) Research Ethics Board (REB) approval for the study for a period of 12 months effective from February 19, 2013 – February 19, 2014. Continuation beyond that date will require further review of REB approval.

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.

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 latter document).

Good luck with your investigations.

With best wishes,

[Signatures]

Dr. Bob Hyland
Chair, Research Ethics Board

Dr. Brendan McDowell
Vice-Chair, Research Ethics Board

Dr. Alexandra Jenkins (REB# 09-272)
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>
</tr>
<tr>
<td></td>
<td>Margarine, Becel</td>
<td>1 tbsp</td>
</tr>
<tr>
<td></td>
<td>Cheese, cheddar</td>
<td>3 slices, 10x 10cm x 1cm</td>
</tr>
</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
30 Bond Street, 10th Floor, Queen South Wing
Toronto, ON M5B 1W8     (416) 864-6060 ext 2596
### 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>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</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**

- Ht (cm): ________
- Wt (kg): ________
- BF (%): ________
- Waist:Hip (cm:cm): ________:_______
- SBP/DBP/HR (mmHg:mmHg):
  - Avg: _____/_____ / _____
  - 1. _____/_____ / _____
  - 2. _____/_____ / _____
  - 3. _____/_____ / _____

**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: ________

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 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 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

How many hours of sleep did you have last night? Does this represent a typical amount?  
☐ Yes  ☐ No

______________ hrs

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

---

**Salvia hispanica LOSS Trial**

Subject #: ___________
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>
</tr>
</thead>
<tbody>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
<tr>
<td>Low 1</td>
<td>--------</td>
<td>2--------</td>
<td>3--------</td>
</tr>
</tbody>
</table>

* Salvia hispanica
* LOSS Trial
* Subject #: __________