The Therapeutic Effects of the Combined Use of American Ginseng (Panax Quinquefolius L.) Extract and Korean Red Ginseng (Panax Ginseng C.A. Meyer) Extract in the Management of Type 2 Diabetes Mellitus and Cardiovascular Risk Factors

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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2010

Abstract

Combination therapy has proven to be a popular treatment strategy for tighter diabetes control. Since the preliminary evidence is suggestive of complementary actions of American (AG) and Korean Red Ginseng (KRG) in improving glycemia, this project was designed to investigate the therapeutic potential of AG and KRG in combination.

Following a randomized, double-blind, placebo-controlled parallel design in a population with diabetes at two centres, the combined use of AG and KRG for 12 weeks was safe, but did not significantly affect glycemic control, blood lipids or blood pressure. However, there was a trend toward lower glycated hemoglobin by 0.7% (p=0.1) and office systolic blood pressure by 5 mm Hg (p=0.052) compared to placebo. These findings encourage further investigation of the mechanism and roles of AG, KRG and their effective components. They also highlight limitations in ginseng research and the need to impose strict regulations to facilitate its standardization.
Acknowledgements

I would like to begin by thanking my supervisors, Dr. Vladimir Vuksan and Dr. Alexandra Jenkins, for trusting me with the responsibility of over-seeing this project and others in the clinic. Their support and the experience they have provided means a lot to me and will play a prominent role in my growth as a student moving forward. I would also like to thank my advisory committee members, Dr. Lilian Thompson and Dr. Thomas Wolever, both of whom provided valuable feedback and were a great team in guiding me throughout my graduate studies. I could not have asked for a better combination of expertise.

I would like to thank my colleagues; in particular Elena Jovanovski, who provided both guidance with her knowledge in the field, and reassurance as I was preparing for this study. I was also privileged to meet Christopher Fairgrieve and Lauryn Choleva during the course of this project; we were introduced as colleagues but have become good friends. I am thankful too for all of the volunteers and undergraduate students at the Risk Factor Modification Centre with whom I have had the pleasure of working with.

My sincere thank you goes to my parents, my siblings and my grandfather for their patience in my constant presence and widespread use of space in our home throughout the writing of this thesis. Their support in this was very much appreciated.

Last but certainly not least, I cannot express my thanks enough to all of the study participants without whom this project would not have been possible. Their contribution in time and company, and of course, adherence to the study protocol was invaluable.

Funding for this project was provided by the Canadian Diabetes Association.
# Table of Contents

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

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

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

List of Tables ................................................................................................................... x

List of Figures .................................................................................................................. xii

List of Appendices .......................................................................................................... xiii

List of Abbreviations ...................................................................................................... xiv

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

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

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

  2.1 The Diabetes Epidemic ............................................................................................. 3

    2.1.1 Introduction ........................................................................................................ 3

    2.1.2 Pathophysiology ............................................................................................... 3

      2.1.2.1 Impaired Insulin Sensitivity ....................................................................... 4

      2.1.2.2 Reduced Insulin Secretion ......................................................................... 5

      2.1.2.3 Increased Hepatic Glucose Output ................................................................. 5

    2.1.3 Evaluating Insulin Sensitivity and Insulin Secretion .......................................... 6

    2.1.4 Glycemic Targets and Treatment ................................................................. 9

    2.1.5 Complementary and Alternative Medicine ..................................................... 10
2.2 Ginseng- “The King of Herbs”: Background

2.2.1 Ginseng History

2.2.2 Botanical Classification, Physical Properties and Life Cycle

2.2.3 Chemical Composition

2.2.3.1 Ginsenosides

2.2.3.1.1 Structure and Nomenclature

2.2.3.1.2 Biosynthesis and Metabolism

2.2.3.1.3 Compositional Variability

2.3 The Therapeutic Potential of Ginseng

2.3.1 An Introduction to American and Korean Red Ginseng

2.3.2 The Glycemia-Lowering Effects of Ginseng

2.3.2.1 Preclinical Evidence

2.3.2.2 Clinical Evidence

2.3.2.2.1 American Ginseng

2.3.2.2.2 Korean Red Ginseng

2.3.3 Ginseng and Lipid Metabolism

2.3.3.1 Preclinical Evidence

2.3.3.2 Clinical Evidence

2.3.4 The Vascular Benefits of Ginseng

2.3.4.1 Preclinical Evidence
2.3.4.2 Clinical Evidence ..................................................38

2.3.5 Safety Profile of Ginseng ..............................................39
  2.3.5.1 Blood Pressure ..................................................39
  2.3.5.2 Hypoglycemia ..................................................40
  2.3.5.3 A Summary of the Evidence .....................................41

Chapter 3: Project Overview ..................................................42
  3.1 Rationale .............................................................42
  3.2 Objective ............................................................43
  3.3 Hypothesis ...........................................................44
  3.4 Study Design ........................................................45

Chapter 4: Materials and Methods ...........................................46
  4.1 Ginseng Preparation ..................................................46
    4.1.1 Ginsenoside Analysis ............................................46
  4.2 Wheat Bran Preparation ..............................................49
  4.3 Recruitment and Subject Selection ..................................49
    4.3.1 Power Analysis ..................................................49
    4.3.2 Recruitment and Screening ....................................49
    4.3.3 Subject Selection and Randomization .........................50
  4.4 Intervention ........................................................52
  4.5 Study Protocol and Timeline ........................................52
4.6 Study Measurements

4.6.1 Blood Samples

4.7 Analytical Procedures

4.7.1 Glycemic Parameters

4.7.1.1 Biochemical Parameters

4.7.1.2 Oral Glucose Tolerance Test

4.7.2 Lipid Parameters

4.7.3 Low-Grade Body Inflammation

4.7.4 Parameters of the Blood Pressure Waveform

4.7.4.1 Office Blood Pressure

4.7.4.2 Arterial Stiffness and Central Blood Pressure

4.7.4.3 Ambulatory Blood Pressure

4.7.5 Safety Measures

4.7.5.1 Biochemical Parameters

4.7.5.2 Questionnaires

4.7.6 Compliance

4.7.6.1 Supplement Consumption

4.7.6.2 Anthropometric Assessment

4.7.6.3 Diet Analysis

4.7.7 Statistical Analysis
Chapter 5: Results

5.1 Study Participants

5.2 Treatment of Missing Data

5.3 Efficacy of Combined AG and KRG on Glycemia

5.3.1 Glycated Hemoglobin

5.3.2 Effect on Secondary Outcome Measures

5.3.2.1 Fasting Blood Glucose and Insulin

5.3.2.2 Postprandial Glycemia

5.4 Efficacy of Combined AG and KRG on Lipid Parameters

5.5 Efficacy of Combined AG and KRG on Low-Grade Body Inflammation

5.6 Efficacy of Combined AG and KRG on Blood Pressure and Augmentation Index

5.6.1 Office Blood Pressure

5.6.2 Arterial Stiffness

5.6.2.1 Peripheral Augmentation Index

5.6.2.2 Central Augmentation Index

5.6.3 Central Blood Pressure

5.6.4 Ambulatory Blood Pressure

5.7 Safety

5.7.1 Symptoms Report

5.7.2 Biochemical Analysis
Chapter 6: Discussion and Conclusions

6.1 An Overview of the Results

6.2 Study Limitations

6.2.1 Sample Size

6.2.2 Data Analysis

6.3 The Yin and Yang Actions of Ginseng

6.4 An Ineffective Ginseng Formulation

6.5 Caveats of Ginseng Research

6.5.1 Complex Composition

6.5.2 Variability in Ginseng Composition and Analytical Methodology

6.6 Future Directions

6.6.1 Standardization of Ginseng

6.6.2 Future Studies

6.7 Conclusion

References

Appendices
List of Tables

2-1 The Botanical Classification of Ginseng ................................................................. 12

2-2 Identified Species of the Genus Panax ................................................................. 13


2-4 In Vitro Studies Examining The Glycemic Effects of Ginseng ........................................ 22

2-5 Preclinical Studies Examining the Glycemic Effects of Ginseng in Animal Models .... 24

2-6 Clinical Studies Investigating the Glycemic Effects of American Ginseng ............ 32

2-7 Clinical Studies Investigating the Glycemic Effects of Korean Red Ginseng ............ 34

4-1 The Ginsenoside Profiles of the Investigational Study Material: American Ginseng and Korean Red Ginseng ................................................................. 48

4-2 Protocol of Study Measurements ........................................................................... 54

4-3 Biochemical Testing Conducted at Core Lab, St. Michael’s Hospital ................. 56

5-1 Distribution of Completed Subjects Between Study Arms and Centres ............... 67

5-2 Baseline Subject Characteristics Comparing Treatment Groups ......................... 68

5-3 Baseline Subject Characteristics Comparing Centres ........................................... 70

5-4 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Lipid Parameters ................................................................. 78

5-5 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Office Blood Pressure ................................................................. 81

5-6 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Central Augmentation Index and Central Blood Pressure ........................................ 82
5-7 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on 24 hour Ambulatory Blood Pressure

5-8 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Kidney and Liver Function

5-9 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Bleeding Time

5-10 Comparing Anthropometric Changes Between Treatment Groups

5-11 Comparing Changes in Diet Composition Between Treatment Groups

6-1 Comparing the Ginsenoside Profile for Ginseng in the Current Investigation to Ginseng used in Previous Investigations

6-2 Comparing the Ginsenoside Profile for American and Korean Red Ginseng as Determined by Different Analysis Methods
List of Figures

2-1 The Ginseng Life Cycle.................................................................................................14
2-2 The Chemical Structure of Protopanaxadiol and Protopanaxatriol Ginsenosides........17
2-3 The Insulin Secretory Pathway....................................................................................26
2-4 The Insulin Signaling Pathway...................................................................................28
4-1 Study Timeline.............................................................................................................53
5-1 Subject Flow at Centre One.......................................................................................66
5-2 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on HbA1c.....................................................................................................................73
5-3 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Fasting Blood Glucose...........................................................................................................75
5-4 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Fasting Blood Insulin..............................................................................................................76
5-5 The Effect of Combined American and Korean Red Ginseng Compared to Placebo on Postprandial Glycemia............................................................................................................77
5-6 The Effect of Combined AG and KRG Compared to Placebo on Office Systolic Blood Pressure........................................................................................................................................80
List of Appendices

Appendix 1 Telephone Screening Questionnaire .......................................................... 116

Appendix 2 Informed Consent Form ............................................................................. 117

Appendix 3 Medical Information Form ........................................................................ 119

Appendix 4 Health Canada Notice of Authorization ................................................... 125

Appendix 5 St. Michael’s Hospital Research Ethics Board Original Approval ............. 126

Appendix 6 University of Toronto Research Ethics Approval ..................................... 129

Appendix 7 Clinical Assessment Form ......................................................................... 130

Appendix 8 Symptoms Questionnaire .......................................................................... 131

Appendix 9 Three Day Food Record ............................................................................ 132
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>ABC</td>
<td>American Botanical Council</td>
</tr>
<tr>
<td>AG</td>
<td>American ginseng</td>
</tr>
<tr>
<td>AI</td>
<td>Augmentation index</td>
</tr>
<tr>
<td>AMBP</td>
<td>Ambulatory blood pressure</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated partial thromboplastin time</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BF</td>
<td>Body fat</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>CAM</td>
<td>Complementary and alternative medicine</td>
</tr>
<tr>
<td>CDA</td>
<td>Canadian Diabetes Association</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatinine</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DAD</td>
<td>Diode array detector</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>FBG</td>
<td>Fasting blood glucose</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<td>----------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>FSIVGTT</td>
<td>Frequent sampling intravenous glucose tolerance test</td>
</tr>
<tr>
<td>GEP</td>
<td>Ginseng evaluation program</td>
</tr>
<tr>
<td>GLUT</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycated hemoglobin</td>
</tr>
<tr>
<td>HDL-C</td>
<td>High-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>HOMA</td>
<td>Homeostatic model assessment</td>
</tr>
<tr>
<td>HPLC-UV</td>
<td>High performance liquid chromatography-ultraviolet spectrophotometry</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High-sensitivity C-reactive protein</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>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>IRS</td>
<td>Insulin receptor substrate protein</td>
</tr>
<tr>
<td>IS</td>
<td>Insulin sensitivity</td>
</tr>
<tr>
<td>ISI</td>
<td>Insulin sensitivity index</td>
</tr>
<tr>
<td>IVGTT</td>
<td>Intravenous glucose tolerance test</td>
</tr>
<tr>
<td>JNK</td>
<td>c-Jun Nh₂-Terminal kinase</td>
</tr>
<tr>
<td>KRG</td>
<td>Korean red ginseng</td>
</tr>
<tr>
<td>LDL-C</td>
<td>Low-density lipoprotein cholesterol</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean arterial pressure</td>
</tr>
</tbody>
</table>
MAPK  Mitogen-activated protein kinase
NEFA  Nonesterified fatty acids
NFKB  Nuclear factor kappa B
NG    Normoglycemic
NO    Nitric oxide
NSAID Non-steroidal anti-inflammatory drugs
OGGA  Ontario ginseng grower’s association
OGTT  Oral glucose tolerance test
PG    Plasma glucose
PI    Plasma insulin
PI3K  Phosphatidylinositol-3-kinase
PP    Pulse pressure
PPG   Postprandial glycemia
PPD   Protopanaxadiol
PPT   Protopanaxatriol
PT    Prothrombin time
QUICKI Quantitative insulin sensitivity check index
RCT   Randomized controlled trial
Rb1   \[2\text{-O-\(\beta\)-glucopyranosyl-(3\(\beta\),12\(\beta\))-20-[(6\text{-O-}\(\beta\)-D-glucopyranosyl-\(\beta\)-D-glucopyranosyl)oxy]-12\text{-hydroxydammar-24-en-3-yl-\(\beta\)-D-glucopyranoside}\]
Rb2   \[20-[(6\text{-O-}\(\alpha\)-L-arabinopyranosyl-\(\beta\)-D-glucopyranosyl)oxy]-12\(\beta\)-hydroxydammar-24-en-3\(\beta\)-yl 2-O-\(\beta\)-D-glucopyranosyl-\(\beta\)-D-glucopyranoside\]
**Rc** 20-[(6-O-α-L-arabinofuranosyl-β-D-glucopyranosyl)oxy]-12β-hydroxydammar-24-en-3β-y1 2-O-β-D-glucopyranosyl-β-D-glucopyranoside

**Rd** 2-O-β-D-glucopyranosyl-(3β,12β)-20-(β-D-glucopyranosyloxy)-12-hydroxydammara-24-en-3-yl-β-D-glucopyranoside

**Re** 2-O-(6-deoxy-α-L-mannopyranosyl)-(3β,6α,12β)-20-(β-D-glucopyranosyloxy)-3,12-dihydroxydammar-24-en-6-yl-β-D-glucopyranoside

**Rf** (3β,6α,12β)-3,12,20-trihydroxydammar-24-en-6-y1 2-O-β-D-glucopyranosyl-β-D-glucopyranoside

**Rg1** (3β,6α,12β)-3,12-dihydroxydammar-24-ene-6,20-diylbis-β-D-glucopyranoside

**Rg3** (3β,12β)-12,20-dihydroxydammar-24-en-3-yl 2-O-β-D-glucopyranosyl-β-D-glucopyranoside

**Rh2** 20-[(6-O-α-L-arabinopyranosyl-β-D-glucopyranosyl)oxy]-12β-hydroxydammar-24-en-3β-y1 2-O-β-D-glucopyranosyl-β-D-glucopyranoside

**Rx** Conventional denomination of ginsenosides

**SBP** Systolic blood pressure

**SD** Standard deviation

**SEM** Standard error of the mean

**T2DM** Type 2 diabetes mellitus

**TC** Total cholesterol

**TCM** Traditional Chinese medicine

**TG** Triglycerides

**T1D** Thrice per day

**WHO** World Health Organization

**WHR** Waist-to-hip ratio

**Wt** Weight
1.1 Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous disorder characterized by elevated blood glucose levels (1,2). The major therapeutic goals are to optimize blood glucose control, blood lipid levels and blood pressure in order to reduce the risk of developing cardiovascular disease (CVD), the cause of approximately 50% of deaths among individuals with T2DM (3-5). Only 13% of individuals diagnosed with T2DM are able to concurrently meet target levels for these goals, emphasizing the need for new, effective treatment options which can be safely used in combination with existing medication (6).

Interest in the use of complementary and alternative medicine for improving glycemic control in T2DM is increasing despite insufficient evidence for its safety and efficacy (7). Ginseng is one of the most popular herbs and one which has demonstrated therapeutic potential in the management of this disease (7). Although ginseng use dates back thousands of years in traditional Chinese medicine, research has only just begun elucidating its physiological actions (8). The use of ginseng to improve glycemic control in individuals with T2DM has been studied for over a decade. Preliminary findings demonstrate that both American Ginseng (AG) and Korean Red Ginseng (KRG) can affect postprandial glycemia acutely and may have long-term effects when used in addition to conventional medication for the treatment of T2DM (9). Although the exact mechanism of action is unknown, preliminary evidence suggests that both species of ginseng act through a different, but complementary mechanism of action; AG may act by increasing insulin secretion, whereas, KRG may act by improving insulin sensitivity (9-11). Since T2DM can include both insulin deficiency and insulin insensitivity as its main
abnormalities, these two species of ginseng used in combination may offer possible therapeutic potential in the management of diabetes and risk factors of CVD (12).

The current study investigates whether the use of AG and KRG in combination is effective and safe in the long-term treatment of T2DM. The study follows a randomized, double-blind, placebo-controlled, two-centre, two-arm parallel design in individuals with T2DM. Effects on glycemic control are assessed by examining biochemical markers and the glycemic response to an oral glucose tolerance test. Additional metabolic parameters and blood pressure parameters, such as central blood pressure and arterial stiffness, are also evaluated as markers of CVD risk.
Chapter 2
Literature Review

The following review will examine ginseng and its potential therapeutic benefits as an herb form of complementary and alternative medicine in the context of the growing incidence of diabetes.

2.1 The Diabetes Epidemic

2.1.1 Introduction

Diabetes is undoubtedly a major health burden affecting more than three million Canadians and nearly 300 million individuals globally (1,3,13). It is considered a growing epidemic in Canada, and a pandemic worldwide (1,3,13). Over 90% of individuals with diabetes are diagnosed with type 2 diabetes mellitus (T2DM), a progressive metabolic disorder characterized by chronic hyperglycemia that is associated with impaired insulin sensitivity, relative insulin deficiency and continued hepatic glucose output (2,3,14-16).

2.1.2 Pathophysiology

The etiology of T2DM is multifactorial and therefore highly complex. While the following discussion emphasizes its main abnormalities, this brief overview cannot be considered a comprehensive review of the subject.

In T2DM, the decreased ability of target tissues to respond to normal insulin levels stimulates β-cells of the pancreatic islets of Langerhans to increase insulin production following a glycemic
stimulus, resulting in hyperinsulinemia (17). Chronic hypersecretion of insulin may occur until β-cell function declines (16,18,19). β-cell exhaustion then leads to a fall in insulin levels while hepatic glucose production continues, due in large part to hepatic insulin insensitivity, resulting in hyperglycemia (15,18,19). Ultimately, β-cell function is unable to compensate for insulin insensitivity as the disease progresses. Most individuals with T2DM are therefore both insensitive to insulin and relatively insulin deficient (14).

2.1.2.1 Impaired Insulin Sensitivity

Reduced insulin-mediated glucose uptake in hepatic and peripheral tissue, or insulin insensitivity, manifests itself as hyperglycemia (17). Although 75% of insulin-stimulated glucose uptake occurs in skeletal muscle, the contribution of hepatic uptake is also critical to insulin insensitivity because hepatic uptake of glucose inhibits glucose production by the liver (2,17).

Controversy surrounds the origin of hepatic as compared to peripheral insulin insensitivity in the pathogenesis of T2DM (17). Hepatic insensitivity was once considered to be a response to peripheral insensitivity; yet recent evidence suggests that hepatic dysfunction may actually occur earlier and contribute to the onset of hyperglycemia (17). The biochemical pathway responsible for insulin action and its dysfunction as associated with T2DM is further discussed in section 2.3.2.1.
2.1.2.2 Reduced Insulin Secretion

Diabetes leads to the damaging of β-cells in the endocrine tissue of the pancreas, whose primary function is to synthesize and secrete insulin—an anabolic hormone responsible for cellular glucose uptake and suppression of hepatic glucose production (20). Insulin secretion normally occurs at a basal rate but β-cell insulin output is increased in response to a rise in blood glucose (2,15,19).

Glucose-stimulated insulin secretion occurs as a biphasic response. The first phase is rapid and results in a burst of insulin, while the second phase is relatively prolonged and persists for the duration of the glycemic stimulus (15,19). Loss of the first phase response has been implicated as an early manifestation of T2DM and can result in continued hepatic glucose output (15). Ultimately, the progression of T2DM can lead to glucotoxicity and oxidative stress, both of which can damage β-cells and induce apoptosis, leading to fewer β-cells and decreased production and secretion of insulin (2,21,22). Dysfunction of the insulin secretory pathway is further discussed in section 2.3.2.1.

2.1.2.3 Increased Hepatic Glucose Output

In the fasting state, blood glucose levels are determined by glucose production and utilization (17). For a normoglycemic individual in the fasting state, the liver supplies 90% of the glucose to both insulin sensitive and insensitive tissues via gluconeogenesis and glycogenolysis (17,23). Normally, basal insulin levels can suppress hepatic glucose output by 60-90%, and an increase in insulin levels following meal consumption acts to further inhibit hepatic glucose output (17,24).
However, with hepatic insulin insensitivity and low levels of insulin, as in the case of insulin deficiency in T2DM, hepatic glucose output continues, resulting in hyperglycemia (15,17).

Hepatic glucose production is regulated not only by the direct actions of insulin on the liver, but also via free fatty acid- and glucagon-mediated actions of insulin (25,26). Glucagon, a hormone released by the \( \alpha \)-cells of the pancreatic islets of Langerhans, counteracts the effect of insulin by increasing glucose levels (26). Suppression of glucagon, and lower levels of nonesterified fatty acids (NEFA) have been implicated in the suppression of hepatic glucose output (25,26,26-29). However, irregular glucagon secretion and elevated NEFA levels are associated with T2DM and have also been observed to contribute to abnormal hepatic glucose output (15,20,25-29).

2.1.3 Evaluating Insulin Sensitivity and Insulin Secretion

Although biochemical assessment methods of insulin sensitivity and insulin secretion vary in the literature, the hyperinsulinemic euglycemic clamp and hyperglycemic clamp are considered the ‘gold’ standards, respectively. These methods entail manipulating the rate of intravenous infusion of glucose and insulin (30). The hyperinsulinemic euglycemic method involves fixing insulin levels at a high concentration while glucose is simultaneously infused to maintain constant plasma glucose levels (30,31). The rate of glucose infusion serves as a measure of glucose uptake in response to exogenous insulin, and is therefore a measure of insulin sensitivity (30). The hyperglycemic clamp, on the other hand, involves fixing glucose at a high concentration (30). The rate of glucose infusion is used as an indicator of glucose metabolism resulting from endogenous insulin production (30).
A variety of factors, including convenience, budget and patient preference, play an important role in the assessment method selected for use in clinical trials. Upon consideration of these factors, these reference methods are not always employed. Alternative methods, such as mathematical models and glucose tolerance tests are often used. They have been validated against these ‘gold’ standard references demonstrating a high level of correlation and can provide insight not only into whole-body insulin sensitivity, but into insulin secretion as well (32).

While the reference method evaluates whole-body insulin sensitivity, insulin sensitivity is often reported using tools that assess either hepatic glucose uptake or peripheral glucose uptake. Mathematical models such as the homeostasis model assessment (HOMA) and the quantitative insulin sensitivity check index (QUICKI) use fasting plasma glucose and insulin levels in the following equations as a determinant of insulin sensitivity (31):

\[
\text{HOMA-IS} = \frac{22.5}{[\text{fasting glucose (mmol/L)} \times \text{fasting insulin (µU/mL)}]} \quad (31)
\]

\[
\text{QUICKI} = \frac{1}{[\log (\text{fasting glucose (mg/dL)}) + \log (\text{fasting insulin (µU/mL)})]} \quad (31)
\]

Since these models are based on fasting values, they primarily evaluate hepatic insulin sensitivity (31).

Modifications to these models have been created to help account for physiological conditions, such as variations in hepatic and peripheral sensitivity, as is the case for the updated HOMA - a computer model (32). A modified version of the QUICKI model also exists and includes the logarithm of fasting NEFA levels in the denominator of the formula in order to improve its association with insulin sensitivity in nonobese individuals (33).
Additional models of evaluating insulin sensitivity include the intravenous glucose tolerance test (IVGTT) and the frequent sampling intravenous glucose tolerance test (FSIVGTT) which utilize minimal model analysis to assess plasma glucose disappearance (31,34).

\( \beta \)-cell function, an indicator of insulin secretion can also be determined using the minimal model and intravenous protocols (34). The area under the insulin response curve post-glucose administration serves to quantify insulin secretion in this case. Similarly, insulin secretion can be quantified using a HOMA model to assess \( \beta \)-cell function (32). Much like the model designed to evaluate insulin sensitivity, it is based on fasting plasma glucose and insulin levels:

\[
\text{HOMA-}\%\beta = \frac{\left(20 \times \text{fasting insulin (}\mu\text{U/mL})\right)}{\left[\text{fasting glucose (mmol/L)} - 3.5\right]} \tag{32}
\]

Biochemical assessment methods evaluating peripheral glucose uptake such as the oral glucose tolerance test (OGTT) are also quite common (17). The simplicity of the OGTT, its clinical utility as a diagnostic tool for determination of impaired glucose tolerance and T2DM, and its physiological relevance provide a solid rationale for its use in clinical settings (17,31,34). This test examines fasting blood glucose levels and the two hour glycemic response to a 75g oral glucose load, both diagnostically critical points in glycemia (1). Mathematical models can assist in the clinical interpretation of the OGTT. Particularly, the insulin sensitivity index (ISI) as calculated using fasting and mean OGTT plasma glucose (PG) and plasma insulin (PI) levels (35).

\[
\text{ISI} = \frac{10000}{\sqrt{\left[\text{fasting PG (mg/dl)} \times \text{fasting PI (}\mu\text{U/ml})\right] \times \left[\text{mean OGTT PG (mg/dl)} \times \text{mean OGTT PI (}\mu\text{U/ml})\right]}} \tag{35}
\]

The ISI models whole-body insulin sensitivity and is therefore considered a better assessment tool for insulin sensitivity (35). Mathematical models for evaluating insulin secretion, such as the
insulinogenic index (II), can also be used in conjunction with the OGTT (36,37). This model provides insight into the first phase insulin response (37).

\[
II = \left[ \frac{30 \text{ minute insulin (\mu U/L)} - \text{fasting insulin (\mu U/L)}}{30 \text{ minute glucose (mmol/L)} - \text{fasting glucose (mmol/L)}} \right]
\] (37,38)

2.1.4 Glycemic Targets and Treatment

The Clinical Practice Guidelines released by the Canadian Diabetes Association (CDA) in 2008 recommended a target of \(\leq 7.0\%\) for glycated hemoglobin (HbA1c), a marker of long-term glycemic control (1,39). Despite these recommendations, the National Health and Nutrition Examination Survey revealed that although glycemic control is improving, more than 40\% of individuals diagnosed with diabetes still fail to meet this target (6). Failure to meet target levels can lead to detrimental health effects including microvascular complications, such as retinopathy, nephropathy and neuropathy, and macrovascular complications, such as cardiovascular disease (CVD) and stroke (15,39,40). The implications of such complications are highlighted in the Heart Outcomes Prevention Evaluation study in which CVD-related events were responsible for greater than 40\% of deaths among individuals with diabetes (41).

Accordingly, the primary goal of diabetes management is to attain glycemic control. Fortunately, there are several treatment options available. First line therapy involves lifestyle modifications including a healthy diet as recommended by the CDA along with a regimen of regular exercise (1,14). However, failure to meet glycemic targets may necessitate the use of second line therapy; medications most often in the form of oral antihyperglycemic agents (14,42). Some of the main classes of these agents include insulin sensitizers, insulin secretagogues, suppressors of hepatic
glucose production, and agents that slow carbohydrate absorption (42). The insulin secretagogue, sulphonylurea, and the insulin sensitizer, metformin, have demonstrated the greatest reductions in HbA1c, lowering HbA1c by 1.5-2.0% (1,42). However, as β-cell function is further exhausted, insulin therapy may eventually be required in lieu of or in addition to oral therapy in order to attain metabolic control (12).

Despite the availability of these options, each is not without its limitations; lifestyle modifications are difficult to make and maintain, and the use of medication is frequently associated with undesired side effects such as hypoglycemia and weight gain (14,42-44). In fact, hypoglycemia is most commonly caused by the use of medication (45). These factors lead patients to experiment with forms of complementary and alternative medicine (CAM), often without notifying their physicians (46).

2.1.5 Complementary and Alternative Medicine

CAM has become a therapeutic option of increasing interest over the last decade and includes various herbs, vitamins and minerals that have shown potential as antidiabetes therapies and have therefore been marketed as such. Since one in three North Americans with diabetes use CAM either alone or in combination with their medications; and because their popularity is growing despite insufficient evidence supporting their safety and efficacy, research in this field is essential (1,46). A 2003 systematic review of 45 different types of CAM used in diabetes therapy deemed that the evidence supporting the efficacy of ginseng, an herb, is amongst the strongest (7).
2.2 Ginseng- “The King of Herbs”: Background

2.2.1 Ginseng History

Found in the mountainous regions of Manchuria, China, the ginseng root, which is believed to have been initially consumed for its nutritional value, is widely revered for its medicinal properties (47). Ginseng is known as an ‘adaptogen’ whereby its use is believed to be related to overall wellbeing and is helpful in response to physical, chemical and biological stress. (8,47-51). It has been in use for several thousand years as part of traditional Chinese medicine (TCM). In Canada, the use of ginseng dates back to 1716 when convinced that the plant could be found in the forested regions of French Canada, which resembled the environment in the mountains of Manchuria, a Jesuit priest working among the Iroquois discovered it growing near the St. Lawrence River (52). The First Nations soon also valued the herb for its medicinal qualities and it became significant to the Canadian economy playing a key role in international trade (52,53).

2.2.2 Botanical Classification, Physical Properties and Life Cycle

Ginseng is a perennial plant indigenous to both Asia and North America (51). It belongs to the plant kingdom under which it is taxonomically organized with the family ‘panax’ (Table 2-1) which consists of 13 species of ginseng that have been identified to date (Table 2-2) (50,54-56). Each species has distinct physical and chemical features that are used in its identification (54,57).

Morphologically, the ginseng plant is composed of a root, short upright rhizomes, stems, leaves, berries and buds, which blossom into flowers after three years (Figure 2-1) (54,57). It is a self-
Table 2-1. The botanical classification of the common herb, ginseng (54).

<table>
<thead>
<tr>
<th>TAXONOMIC RANK</th>
<th>CLASSIFICATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Embryophyta Siphonogama</td>
</tr>
<tr>
<td>Subphylum</td>
<td>Angiospermae</td>
</tr>
<tr>
<td>Class</td>
<td>Dicotyledoneae</td>
</tr>
<tr>
<td>Subclass</td>
<td>Archichlamydeae</td>
</tr>
<tr>
<td>Order</td>
<td>Umbelliflorae</td>
</tr>
<tr>
<td>Family</td>
<td>Araliaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Panax</td>
</tr>
</tbody>
</table>
Table 2-2. The thirteen identified species of the genus *Panax* (56).

<table>
<thead>
<tr>
<th>PANAX</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Panax ginseng</em></td>
<td><em>Panax stipuleanatus</em></td>
<td><em>Panax wangianus</em></td>
</tr>
<tr>
<td><em>Panax quinquefolius</em></td>
<td><em>Panax bipinnatifidus</em></td>
<td><em>Panax major</em></td>
</tr>
<tr>
<td><em>Panax japonicus</em></td>
<td><em>Panax omeiensis</em></td>
<td><em>Panax sinensis</em></td>
</tr>
<tr>
<td><em>Panax trifolius</em></td>
<td><em>Panax zingiberensis</em></td>
<td><em>Panax pseudoginseng</em></td>
</tr>
<tr>
<td><em>Panax notoginseng</em></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2-1. The ginseng life cycle lasts between three and six years. Roots and rhizomes grow underground during this time while stems, leaves, and buds grow above ground during the spring and summer months only. Flowers bloom during the third year and seeds are generated from berries which form during the fourth year (61) Drawing from Growing & Marketing Ginseng, Goldenseal & Other Woodland Medicinals, used by permission. Copyright 2005. Bright Mountain Books, Fairview, NC.
pollinating plant whose roots grow underground year-round, while the aerial growth terminates in the fall and resumes in the spring (54). Ginseng is normally harvested between three and six years of age, which is considered a measure of product quality in the Asian market (51,58). Species can be differentiated by physical attributes including the shape of the root and leaflets, and the number of leaves and flowers (57).

2.2.3 Chemical Composition

The ginseng plant contains several hundred components, making it difficult to identify a single agent responsible for its efficacy (59). Its chemical constituents include peptides, fatty acids, vitamins and minerals (60). However, it is the triterpenoid saponins referred to as ginsenosides, the polysaccharides and the polyacetylenes that are considered the active components of ginseng. (50). Of these, the ginsenosides are considered the most pharmacologically active and are consequently the main focus of ginseng research (51,60).

2.2.3.1 Ginsenosides

2.2.3.1.1 Structure and Nomenclature

More than 150 ginsenosides have been isolated from the roots, stems, leaves and flowers of the ginseng plant (50). They are amphiphilic glycosides belonging to the steroid family of molecules, and classified based on their aglycone, known as a sapogenin, which can be of the dammarane- or oleanane-type (50,51). The dammarane-type ginsenosides are further divided into protopanaxadiols (PPD), protopanaxatriols (PPT) and ocolitlol ginsenosides (50). Despite the
existence of numerous ginsenosides, only six in the PPD and PPT groups are the focus of research as it has been suggested that they make up greater than 90% of the ginsenoside composition of the ginseng root, though this remains controversial (49,62). The extensive list of ginsenosides can be attributed to the ensuing variability from different type, number and attachment sites of sugar moieties to the hydroxyl groups on the aglycone backbone (Figure 2-2) (50,63).

Ginsenosides are generally designated as Rx where ‘x’ distinguishes ginsenosides from one another by a different letter of the alphabet, which may be followed by a numerical value (50). They were initially named based on their polarity, as determined by their mobility on a thin-layer chromatography plate (60). The value of their retention factor corresponded with their sequence on the plate from bottom to top (a to h) and, in turn, their alphabetical designation (64,65). Nevertheless, due to the large number of ginsenosides discovered to date, this form of nomenclature is no longer used (50). Current strategies for naming newly discovered ginsenosides can include reference to the species and part of the plant from which they were isolated (50). Nomenclature may also be based on the sequence of ginsenoside identification and derivation from ginsenosides similar in structure but with differences in functional groups recognized (50,66).
Figure 2-2. Two of the main classes of ginsenosides found in ginseng are the PPDs and PPTs. Ginsenosides differ in their type, number and attachment site of sugar moieties (R) to the hydroxyl groups on carbons 3, 6 and 20 of the aglycone backbone.
2.2.3.1.2 Biosynthesis and Metabolism

Ginsenosides are synthesized in the cytosol of the plant cell via the isoprenoid pathway with mevalonic acid as a precursor, leading to the formation of 2,3-oxidosqualene (50). Cyclization of 2,3-oxidosqualene generates the triterpenoid backbone and subsequent hydroxylation, oxidation, substitution and glycosylation reactions lead to the formation of the >150 known ginsenosides (50).

Although studies examining the pharmacokinetic and pharmacodynamic properties of ginsenosides are few, it has been demonstrated that ginsenosides of the PPT class are first hydrolyzed by gastric pH, while the PPD ginsenosides evade digestion in the stomach (67). Further metabolism of both PPDs and PPTs is carried out by the colonic microflora which metabolize ginsenosides to their monoglycosylated forms and their hydrated products (67). PPDs are metabolized to compound K and the PPD aglycone, while PPTs are metabolized to ginsenosides Rh1, F1 and the PPT aglycone (67).

The pharmacokinetic time course of ginsenoside metabolism varies depending on the ginsenoside examined. This was demonstrated through the analysis of blood plasma and urine samples from two healthy individuals following oral ingestion of ginseng capsules (67). PPT metabolites began appearing in plasma after 1-3 hours although PPD metabolites did not appear until 5.5-8 hours following ginseng ingestion (67). Ginsenosides could not be detected in plasma beyond 12 hours and the bioavailability of the mono- and deglycosylated ginsenosides was demonstrated to be greater than that of the intact ginsenoside (50,67). Urine analysis revealed that the PPDs and PPTs that were not identified in the first few hours of the plasma analysis began appearing in urine between zero and three hours post-ingestion. This suggests that they are absorbed, metabolized, distributed and excreted relatively quickly. Complete elimination of
ginsenoside metabolites appeared to occur within one day as they could not be detected in urine beyond 24 hours post-ingestion (67). Nevertheless, the sample size and detection limits of the instruments used in analysis must be considered as limitations in this study and the results interpreted accordingly.

2.2.3.1.3 Compositional Variability

Biological variability of ginsenoside composition exists both between and within ginseng species and interspecies variability plays an important role in species identification as will be discussed shortly hereafter.

Ginsenoside composition also differs between wild and cultivated products, whereby wild ginseng is more abundant in ginsenoside content (68). Furthermore, the process of cultivation includes many sources of variability. Not only do environmental conditions such as moisture content of soil and exposure to sunlight influence ginsenoside composition, but year of harvest and the part of the plant being investigated (i.e. root, rootlet, leaves, berries) all lead to compositional differences (69,70). Varying extraction and processing methods are subsequent sources of variability. The many sources of variability in ginseng production and the ensuing compositional variability of ginseng products are often used to explain the variability in results obtained in ginseng research. Despite these limitations in ginseng research, it remains the top herb used in diabetes therapy (51).
2.3 The Therapeutic Potential of Ginseng

2.3.1 An Introduction to American and Korean Red Ginseng

Of the thirteen known species of ginseng, the two that are the most popularly used and for which there is the most clinical evidence for efficacy are *Panax Quinquefolius* L. known as American Ginseng (AG) and the steamed form of *Panax Ginseng* C.A. Meyer, known as Korean Red Ginseng (KRG). Table 2-3 identifies the compositional differences in the ginsenoside profile between these species (51,54,69,70).

<table>
<thead>
<tr>
<th>AMERICAN GINSENG</th>
<th>KOREAN RED GINSENG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher Total Ginsenosides</td>
<td>Contains Rf and Rg3</td>
</tr>
<tr>
<td>Rb1:Rg1 is &gt;3</td>
<td>Rb1:Rg1 is &lt;3</td>
</tr>
<tr>
<td>Rg1:Re &lt;1</td>
<td>Rg1:Re &gt;1</td>
</tr>
<tr>
<td>Rb2:Rc &lt;1</td>
<td>Rb2:Rc &gt;1</td>
</tr>
</tbody>
</table>

Rf, Rg3, Rb1, Rg1, Re, Rb2, Rc- conventional nomenclature of ginsenosides

Although clinical studies tend to focus on the effect of either ginseng preparations of the whole root or specific parts of the root, as is administered in TCM, preclinical studies try to elucidate the role of individual ginsenosides in its therapeutic efficacy.
2.3.2 The Glycemia-Lowering Effects of Ginseng

2.3.2.1 Preclinical Evidence

The most consistent evidence illustrating the glycemia-lowering potential of ginsenosides and their metabolites comes from in vitro studies and in vivo studies using animal models. Some of the most recent and compelling evidence is outlined in Table 2-4 and Table 2-5.

As indicated in these tables, different doses of ginsenosides and their metabolites, such as compound K, a PPD metabolite, administered to various cell lines and animal models used in diabetes research show a significant lowering of fasting and postprandial glycemia (PPG). This improvement in glycemia generally occurs either through improved insulin sensitivity or enhanced insulin secretion, demonstrating the potential of ginseng as a therapy in diabetes management.

These cell culture and animal studies have also provided insight into possible targets of individual ginsenosides in the insulin secretory and insulin signaling pathways that may explain these glycemia-lowering observations. Prior to describing these effects, it is first necessary to understand the normal physiology of these pathways and their dysfunction as it relates to T2DM.

In the normal pancreatic β-cell (Figure 2-3), glucose enters via facilitated diffusion through protein channels intrinsic to the plasma bilayer known as glucose transporters (2,71). Once inside, glucose undergoes glycolysis generating adenosine triphosphate (ATP), which leads to an increased ratio of ATP to adenosine diphosphate (ADP) (20,72). The change in ATP/ADP concentration triggers the closure of the ATP-sensitive potassium (K⁺) channels resulting in cellular depolarization, which activates the voltage-dependent calcium (Ca²⁺) channels. Activation of these channels results in extracellular Ca²⁺ influx leading to calcium-dependent
Table 2-4. *In vitro* studies examining the effects of ginseng extract, individual ginsenosides and their metabolites on glycemic control in cell lines used in diabetes research.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>GINSENOside</th>
<th>DOSE</th>
<th>CELL TYPE</th>
<th>OUTCOME</th>
<th>SUGGESTED MECHANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007 Chang et al (73)</td>
<td>Rg1</td>
<td>0.01-0.1 μM</td>
<td>Caco-2</td>
<td>↓ glucose absorption across intestinal epithelium</td>
<td>decreased expression of sodium/glucose cotransporter</td>
</tr>
<tr>
<td>2007 Han et al (74)</td>
<td>Compound K</td>
<td>8 μM</td>
<td>HIT T15, Pancreatic Islets from male SD rats</td>
<td>↑ insulin secretion</td>
<td>inhibition of ATP sensitive potassium channels</td>
</tr>
<tr>
<td>2008 Park et al (43)</td>
<td>Rg3</td>
<td>5 μM</td>
<td>HIT T15</td>
<td>↑ glucose-stimulated insulin secretion</td>
<td>activates AMPK pathway which stimulates translocation of glucose transporter</td>
</tr>
<tr>
<td>2008 Zhang et al (75)</td>
<td>Re</td>
<td>10 μM</td>
<td>3T3 L1</td>
<td>↑ glucose uptake</td>
<td>promotes GLUT4 translocation by activating IRS1 independent of IR activation, inhibits JNK and NFκB, activates PI3K</td>
</tr>
<tr>
<td>2008 Shang et al (76)</td>
<td>Rb1</td>
<td>0.01-10 μM</td>
<td>3T3 L1</td>
<td>↑ insulin mediated glucose uptake</td>
<td>promote translocation of GLUT1 and GLUT4 by activating PI3K</td>
</tr>
<tr>
<td>2008 Park et al (77)</td>
<td>Rg1, Rb1</td>
<td>20 μM</td>
<td>MIN 6</td>
<td>↑ glucose-stimulated insulin secretion</td>
<td>PKA activation which lead to increased mRNA and protein expression of IRS2</td>
</tr>
</tbody>
</table>
Table 2-4 Continued

<table>
<thead>
<tr>
<th>STUDY</th>
<th>GINSENOside</th>
<th>DOSE</th>
<th>CELL TYPE</th>
<th>OUTCOME</th>
<th>SUGGESTED MECHANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 Kim and Kim (78)</td>
<td>KRG extract</td>
<td>0.1-1.0 mg/mL</td>
<td>Pancreatic Islets from male Sprague-Dawley rats</td>
<td>↑ insulin secretion</td>
<td>inhibition of ATP sensitive potassium channels</td>
</tr>
<tr>
<td>2008 Park et al (79)</td>
<td>Ginseng Radix extract</td>
<td>50 μg/mL</td>
<td>Pancreatic Islets from male rats</td>
<td>↑ glucose-stimulated insulin secretion</td>
<td>↑ mRNA expression of glucokinase (acts as a glucose sensor)</td>
</tr>
<tr>
<td>2008 Lin et al (80)</td>
<td>AG berry extract, Re</td>
<td>0.1-1.0 mg/mL</td>
<td>MIN 6</td>
<td>↑ insulin secretion</td>
<td>↓ oxidative stress which can lead to insulin resistance</td>
</tr>
<tr>
<td>2010 Kim et al (81)</td>
<td>Rg3</td>
<td>0.1-5.0 μM</td>
<td>MIN 6N8</td>
<td>↓ β-cell apoptosis</td>
<td>prevented palmitate-induced apoptosis</td>
</tr>
</tbody>
</table>

Rg1, Rg3, Re, Rb1- conventional nomenclature of ginsenosides; Compound K- protopanaxadiol metabolite; PPD- protopanaxadiols; KRG- Korean Red Ginseng; AG- American Ginseng; ATP- adenosine triphosphate; AMPK- adenosine monophosphate-activated protein kinase; GLUT4- glucose transporter isoform 4; IRS1- insulin receptor substrate protein 1; IR- insulin receptor; JNK- c-Jun N-terminal kinase; NFκB- nuclear factor kappa B; PI3K- phosphatidylinositol-3-kinase; GLUT1- glucose transporter isoform 1; PKA- protein kinase A; IRS2- insulin receptor substrate protein 2
Table 2-5. *In vivo* studies conducted with diabetes rodent models examining the effects of administration of ginseng extract, ginsenosides and their metabolites.

<table>
<thead>
<tr>
<th>STUDY</th>
<th>GINSENOSIDE</th>
<th>DOSE</th>
<th>ANIMAL MODEL</th>
<th>OUTCOME</th>
<th>SUGGESTED MECHANISM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002 Attele et al (63)</td>
<td>berry extract, Re</td>
<td>150 mg/kg i.p.</td>
<td>ob/ob mice</td>
<td>↓ fasting and postprandial glycemia</td>
<td>↑ insulin-stimulated glucose disposal</td>
</tr>
<tr>
<td>2005 Xie et al (82)</td>
<td>Re</td>
<td>20 mg/kg i.p.</td>
<td>ob/ob mice</td>
<td>↓ fasting and postprandial glycemia</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2005 Liu et al (83)</td>
<td>Panax Ginseng root</td>
<td>125 mg/kg oral</td>
<td>Male Wistar rats</td>
<td>↓ plasma glucose AUC and glucose insulin index</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2006 Lee et al (84)</td>
<td>Rh2</td>
<td>1.0 mg/kg i.v.</td>
<td>male Wistar rats</td>
<td>↓ fasting glycemia</td>
<td>↑ insulin secretion</td>
</tr>
<tr>
<td>2007 Han et al (74)</td>
<td>Compound K (PPD)</td>
<td>10/20 mg/kg oral</td>
<td>ICR and db/db mice</td>
<td>↓ fasting and postprandial glycemia</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2007 Yoon et al (85)</td>
<td>Compound K</td>
<td>10 mg/kg</td>
<td>db/db mice</td>
<td>↓ fasting glycemia</td>
<td>↑ insulin secretion</td>
</tr>
<tr>
<td>2007 Lee et al (86)</td>
<td>Rh2</td>
<td>1.0 mg/kg i.v.</td>
<td>male Wistar rats</td>
<td>↓ fasting glycemia</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2007 Banz et al (87)</td>
<td>root extract</td>
<td>10 g/kg</td>
<td>Male ZDF rats</td>
<td>↓ postprandial glycemia</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2007 Xie et al (88)</td>
<td>AG berry juice</td>
<td>0.6 mL/kg</td>
<td>ob/ob mice</td>
<td>↓ postprandial glycemia</td>
<td>not specific</td>
</tr>
<tr>
<td>STUDY</td>
<td>GINSENOSIDE</td>
<td>DOSE</td>
<td>ANIMAL MODEL</td>
<td>OUTCOME</td>
<td>SUGGESTED MECHANISM</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------------</td>
<td>----------</td>
<td>--------------</td>
<td>--------------------------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>2008 Chen et al (89)</td>
<td>Rg1, Rb1, Rd, Re</td>
<td>50/200 mg/kg</td>
<td>KK-Ay male mice</td>
<td>↓ fasting and postprandial glycemia</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2008 Park et al (43)</td>
<td>Rg3</td>
<td>12.5,25 mg/kg oral</td>
<td>ICR mice</td>
<td>↓ postprandial glycemia</td>
<td>↑ insulin secretion</td>
</tr>
<tr>
<td>2008 Zhang et al (75)</td>
<td>Re</td>
<td>40 mg/kg i.p</td>
<td>Wistar rats</td>
<td>↓ fasting glycemia</td>
<td>↑ insulin sensitivity</td>
</tr>
<tr>
<td>2008 Kang et al (90)</td>
<td>Rg3</td>
<td>5,10,20 mg/kg oral</td>
<td>STZ- rats</td>
<td>↓ serum glucose and glycosylated proteins</td>
<td>inhibit NMDA receptor-mediated nitrosative stress</td>
</tr>
<tr>
<td>2008 Kim et al (91)</td>
<td>KRG extract</td>
<td>100 mg/kg</td>
<td>Male Wistar rats</td>
<td>↓ serum glucose</td>
<td>↓ oxidative stress</td>
</tr>
<tr>
<td>2009 Liu et al (92)</td>
<td>malonyl-ginsenosides</td>
<td>120 mg/kg</td>
<td>STZ-diabetic mice</td>
<td>↓ fasting glycemia</td>
<td>not specified</td>
</tr>
<tr>
<td>2009 Lee et al (93)</td>
<td>KRG</td>
<td>200 mg/kg</td>
<td>OLETF rats</td>
<td>↓ fasting glucose and HbA1c</td>
<td>↑ insulin sensitivity</td>
</tr>
</tbody>
</table>

Re, Rh2, Rg1, Rb1, Rd, Re, Rg3- conventional nomenclature of ginsenosides; Compound K-protopanaxadiol metabolite; PPD- protopanaxadiols; AG- American Ginseng; KRG- Korean Red Ginseng; i.p.- intraperitoneal; i.v.- intravenous; ZDF- Zucker diabetic fatty; STZ- streptozotocin; NMDA- N-methyl-D-aspartic acid
Glucose enters the β-cell via a glucose transporter and is metabolized generating ATP. Increased ATP:ADP leads to the closure of the ATP-sensitive K⁺ channels. The subsequent cellular depolarization activates voltage-dependent Ca²⁺ channels leading to exocytosis of insulin. Image adapted from The UPS in diabetes and obesity (20). The use of this image is by permission from BioMed Central, London, United Kingdom. Copyright 2008.
fusion of insulin granules with the plasma membrane and subsequent exocytosis, increasing insulin levels (20,71,72).

Insulin deficiency in diabetes has been associated with reduced levels of ATP production and open ATP-sensitive K⁺ channels (94,95). In addition, hyperglycemia in diabetes can lead to oxidative stress-induced β-cell apoptosis through destabilization of the mitochondrial membrane (96).

Preclinical data demonstrate that ginsenosides may improve insulin secretion through increased ATP production by upregulating the activity of glycolytic enzymes leading to the production of ATP (60). Ginsenosides have too been implicated in inhibiting the ATP-sensitive K⁺ channels located in the plasma bilayer by binding their sulphonyl receptors, leading to insulin secretion (74,77,78). This demonstrates an insulin secretagogue effect of ginsenosides, much like the conventional drug therapy, sulphonylurea (42,78). Ginsenosides have also been observed to decrease pancreatic β-cell apoptosis, allowing these cells to continue producing and secreting insulin (60,81). This observation is supported by evidence that ginsenosides can inhibit free fatty acid-mediated apoptosis and regulate the activity and expression of pro- and anti-apoptotic factors, such as caspase 9 and Bcl-2, respectively (81,97).

In addition to the insulin secretory pathway, the normal physiology of the insulin signaling pathway must be considered (Figure 2-4). Normally, in adipocytes and muscle cells, insulin binding to its receptor activates the tyrosine kinase activity intrinsic to the β-subunits on the cytoplasmic surface of the receptor (20,98). Activation of the receptor results in autophosphorylation followed by recruitment and phosphorylation of insulin receptor substrate (IRS) proteins (20,98). Subsequently, additional signaling molecules, such as phosphatidyinositol-3-kinase (PI3K), are recruited and phosphorylated leading to the activation
Figure 2-4. The Insulin Signaling Pathway
The binding of insulin to the tyrosine kinase receptor in the plasma bilayer of adipocytes/skeletal muscle cells activates a phosphorylation cascade leading to cell proliferation and metabolic effects including glycogen synthesis. Image adapted from The UPS in diabetes and obesity (20). The use of this image is by permission from BioMed Central, London, United Kingdom. Copyright 2008.
of the Akt signaling pathway, which mediates the metabolic effects of insulin, including glucose uptake and glycogen synthesis (20,99,100). The mitogen-activated protein kinase (MAPK) pathway is also activated, leading to cellular growth and differentiation (20,99).

Impaired insulin action leading to insulin resistance can occur due to defects in the insulin signaling pathway. Specifically, downregulation of the insulin receptor or other signaling proteins involved in this pathway plays a major role in the attenuation of the insulin signal (20,99). Downregulation may result from decreased protein expression or protein activity, or from receptor internalization and degradation (20,99). Serine phosphorylation of critical amino acids of the IRS proteins, that otherwise undergo tyrosine phosphorylation, has also been implicated in insulin insensitivity (98,101). Proinflammatory cytokines play a role in this by activating the enzymes responsible for incorrectly phosphorylating proteins involved in the signaling pathway (98).

Preclinical research has demonstrated that ginsenosides may inhibit enzymes such as c-Jun NH2-Terminal Kinase (JNK) and Nuclear Factor kappa B (NFκB) that are involved in incorrectly phosphorylating the IRS proteins (75). Activation of components of the insulin signaling pathway independent of insulin binding its receptor has also been demonstrated as an effect of ginsenoside administration (76). In addition, ginsenosides have been observed to regulate downstream effects of insulin action; they can increase the expression and translocation of the glucose transporter, GLUT4, to the plasma membrane in order to facilitate glucose uptake (76).

Preclinical studies have not only demonstrated insulin sensitizing and secretory effects of ginsenoside administration, they have also demonstrated an effect on glucose absorption. In a 2007 study by Chang et al, the potential of ginseng to lower glucose levels was demonstrated via reduced expression and activity of the sodium/glucose cotransporter, a protein responsible for
glucose transport across the intestinal epithelium (73). A significant reduction in glucose transport was observed after a Caco-2 cell monolayer, an *in vitro* model of the intestinal epithelium, was pretreated for 24 hours with 0.01 or 0.1 μM Rg1, a PPT ginsenoside, compared to control (73). The effect of Rg1 on intestinal glucose absorption demonstrated its potential to attenuate postprandial glycemia by altering the expression level of the primary glucose transport protein in the intestinal epithelium (73).

In general, preclinical evidence has consistently demonstrated an improvement in glycemic control and provided insight into the pathways by which ginsenosides may exert their effects. However, ginseng is traditionally consumed as a whole root that contains many different ginsenosides and therefore, it must be considered as such.

### 2.3.2.2 Clinical Evidence

Both acute and long-term clinical studies conducted with ginseng have provided insight into the net, or overall, mechanistic effect of the ginseng species studied. They have also demonstrated the glycemia-lowering potential observed in animal studies, thus supporting its clinical relevance. These effects have been demonstrated for both AG and KRG.

#### 2.3.2.2.1 American Ginseng

*Table 2-6* summarizes some clinical studies investigating the effects of AG on glycemic control. Collectively, they illustrate a decrease in PPG in both normoglycemic (NG) individuals and individuals with T2DM treated acutely with AG. Most of the findings highlighted in this table
are part of the acute-to-chronic clinical testing program in which a series of acute studies investigating an effective dose and time of administration were used to select AG material to administer in a long-term study.

The acute studies revealed that there was a significant reduction in PPG by about 15-22% compared to placebo irrespective of dose (1-9g) and time of administration (0 minutes to 2 hours before a meal) in individuals with diabetes (102-105). A significant improvement in incremental area under the glucose curve (iAUC) by 9-39% was also observed for NG individuals. However this effect was time dependent, as administration of ginseng was shown to be effective if taken 40 minutes to 2 hours prior to a meal, but not effective if taken with a meal (104).

An acute study investigating varying AG extracts was carried out to elucidate the preparation of AG that is most effective in improving glycemia. AG whole root and 30%, 50% and 70% ethanol extracts of AG were administered to 13 NG individuals in a crossover design (106). The study failed to demonstrate an effect on the iAUC for glucose and insulin; however, it did show that insulin sensitivity, as calculated using the ISI discussed in section 2.1.3, was significantly improved with the 50% ethanol extract (106).

The overall effect of the AG ginsenoside profile on glycemia was demonstrated in an acute crossover study where eight NG individuals were administered 6g of AG prior to a 75g OGTT (11). The results revealed a significant decrease in postprandial blood glucose levels and a concomitant increase in insulin secretion, suggesting that AG may increase insulin secretion (11).
Table 2-6. Acute and long-term studies conducted in normoglycemic individuals and those with T2DM investigating the dose, time of administration and efficacy of American Ginseng at the Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada).

<table>
<thead>
<tr>
<th>STUDY</th>
<th>SAMPLE SIZE</th>
<th>TREATMENT</th>
<th>iAUC REDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 Vuksan et al (103)</td>
<td>10 NG</td>
<td>3g AG vs. placebo</td>
<td>18%</td>
</tr>
<tr>
<td>2000 Vuksan et al (103)</td>
<td>9 T2DM</td>
<td>3g AG vs. placebo</td>
<td>22%</td>
</tr>
<tr>
<td>2000 Vuksan et al (102)</td>
<td>10 NG</td>
<td>3,6 or 9g AG vs. placebo</td>
<td>26, 29 or 39%</td>
</tr>
<tr>
<td>2000 Vuksan et al (105)</td>
<td>10 T2DM</td>
<td>3,6 or 9g AG vs. placebo</td>
<td>20, 15 or 16%</td>
</tr>
<tr>
<td>2001 Vuksan et al (104)</td>
<td>12 NG</td>
<td>1,2 or 3g AG vs. placebo</td>
<td>14, 15, or 9%</td>
</tr>
<tr>
<td>2007 Dascalu et al (107)</td>
<td>12 NG</td>
<td>9g AG (5 farms) vs. placebo</td>
<td>28%</td>
</tr>
<tr>
<td>2008 De Souza (106)</td>
<td>13 NG</td>
<td>3g whole root, 30%, 50%, 70% AG extract vs. placebo</td>
<td>NS but ↑ ISI with 50% extract</td>
</tr>
<tr>
<td>2001 Vuksan et al (108)</td>
<td>24 T2DM</td>
<td>3g/d AG vs. placebo</td>
<td>10%</td>
</tr>
</tbody>
</table>

NG- normoglycemic; T2DM- Type 2 diabetes mellitus; AG- American Ginseng; iAUC- incremental area under the glucose curve; NS- not significant; ISI; insulin sensitivity index
These findings were further supported by a long-term study with a crossover design conducted in 24 individuals with T2DM who consumed 3g/day of AG for eight weeks. Both fasting blood glucose and HbA1c significantly decreased while there was an increase in insulin. Though not considered statistically significant, this trend of increased insulin levels was similar to that of the acute study (108).

2.3.2.2.2 Korean Red Ginseng

Although less investigated than AG, clinical studies investigating the effects of acute doses of KRG have also demonstrated significant reductions in PPG (Table 2-7). Similar to AG, an acute-to-chronic clinical testing program was also carried out for KRG. In the first part of a two-phase study investigating the efficacy of the different plant components of KRG, rootlets were found to show the greatest reduction in PPG, lowering iAUC for glucose by 29% when compared to placebo (109). These findings were then used in a dose response study in which KRG rootlets were found to be equally effective in reducing PPG, independent of doses between 2-6g, lowering iAUC for glucose by a mean of 17% when compared to placebo (109). These acute studies were used to select the KRG material to administer in a long-term study in which the overall mechanistic effect of KRG was identified.
Table 2-7. Acute and long-term studies conducted in normoglycemic individuals and those with T2DM investigating the dose, different root components and efficacy of Korean Red Ginseng at the Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada).

<table>
<thead>
<tr>
<th>STUDY</th>
<th>SAMPLE SIZE</th>
<th>TREATMENT</th>
<th>iAUC REDUCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006 Sievenpiper et al (109)</td>
<td>7 NG</td>
<td>6g KRG-rootlets, body, H20 extract vs. placebo</td>
<td>29% for KRG rootlets</td>
</tr>
<tr>
<td>2006 Sievenpiper et al (109)</td>
<td>12 NG</td>
<td>2,4,6g KRG vs. placebo</td>
<td>17%</td>
</tr>
<tr>
<td>2008 De Souza (106)</td>
<td>13 NG</td>
<td>3g whole root, 30%, 50%, 70% KRG extract vs. placebo</td>
<td>NS but ↑ ISI with 30% extract</td>
</tr>
<tr>
<td>2008 Vuksan et al (10)</td>
<td>19 T2DM</td>
<td>6g/d KRG vs. placebo</td>
<td>8-11%</td>
</tr>
</tbody>
</table>

NG- normoglycemic; T2DM- Type 2 Diabetes Mellitus KRG- Korean Red Ginseng; iAUC- incremental area under the glucose curve; NS- not significant; ISI; insulin sensitivity index
In this randomized, crossover, placebo-controlled long-term study, 19 individuals with T2DM consumed 6g/day of KRG for 12 weeks (10). This study illustrated a decrease in postprandial blood glucose with a concomitant decrease in insulin, which is opposite to the effect on insulin that was observed for AG (10). Insulin sensitivity, as calculated using the ISI, was found to be significantly improved following a 75g OGTT (10). Fasting insulin levels were also significantly decreased, and in turn fasting IS, once again supporting an insulin sensitizing mechanism (10).

A more recent study investigating the most effective preparation of KRG extract with similar methodology to the aforementioned AG extract finding study, revealed that acute administration of KRG did not significantly affect iAUC for glucose or insulin (106). Nevertheless, ISI was significantly improved following administration of the 30% ethanol extract (106).

2.3.3 Ginseng and Lipid Metabolism

Dyslipidemia is a comorbidity of T2DM and a risk factor in the development of CVD. Since individuals with T2DM have two to four times greater risk of developing CVD compared to NG individuals, the therapeutic potential of ginseng use in improving lipid profile is an important consideration not only in diabetes management, but also in CVD prevention (1).

2.3.3.1 Preclinical Evidence

A preclinical study examining the effect of orally administered 10g AG root extract per kilogram of diet fed to male Zucker diabetic fatty rats for 11 weeks in a randomized, parallel, placebo-controlled study found cholesterol levels to be significantly decreased (87). A separate study
revealed that two weeks of oral administration of 20mg/kg ginsenoside Re to streptozotocin-induced diabetic rats resulted in a significant reduction in total cholesterol and triglycerides when compared to a control treatment (39). The effect on cholesterol was dose dependent and was associated with an improvement in antioxidant efficacy (22,39).

Improvements to lipid profile through ginseng administration have also been associated with activation and increased expression of peroxisome proliferator-activated receptors, transcription factors regulating expression of proteins involved in lipid metabolism and adipocyte differentiation (50,110).

2.3.3.2 Clinical Evidence

Improvements to lipid profiles have been observed as secondary outcomes in long-term studies conducted with AG or KRG in humans. The long-term study in the AG clinical testing program revealed a significant decrease in serum total cholesterol and LDL cholesterol, compared to placebo, with a dose of 1g AG TID (108). Although the long-term study in the KRG clinical testing program failed to show a significant improvement in lipid profile, a 1983 study by Yamamoto et al showed that administration of 1.5g KRG TID significantly decreased triglycerides and increased HDL cholesterol after one week of ginseng administration in five healthy and six hyperlipidemic individuals (111).
2.3.4 The Vascular Benefits of Ginseng

Similar to dyslipidemia, hypertension is a comorbidity of T2DM and a risk factor for the development of CVD. T2DM can damage the endothelial lining and smooth muscle of the arterial system, leading to vasoconstriction and increased arterial stiffness (113-116). Individuals with T2DM are recommended to maintain BP levels less than 130/80 mm Hg; nevertheless, fewer than 15% of Canadians treated with antihypertensive agents attain adequately controlled BP levels (1,112). Although hypertension is conventionally assessed at the brachial artery, additional determinants of CVD risk, such as 24 hour ambulatory blood pressure, central blood pressure and arterial stiffness also have prognostic value and provide additional information about the cardiovascular system (114,117).

Some of the recent evidence demonstrating a therapeutic benefit of ginseng use in vascular function and blood pressure is presented hereafter.

2.3.4.1 Preclinical Evidence

Animal studies have demonstrated that the administration of the ginsenoside fraction of KRG to anesthetized rats reduces mean arterial blood pressure in a dose-dependent manner (118). These hypotensive effects have been associated with vascular endothelial cell-mediated nitric oxide (NO) release, which results in the production of cyclic guanosine monophosphate (cGMP) and subsequent relaxation of smooth muscle (118,119). A separate study found a significant reduction in SBP following administration of KRG in both normotensive and hypertensive rats (119).
Although the composition of KRG is complex, it was the PPTs that were first implicated as the component of KRG responsible for its improvement of vascular tone (120). They demonstrated greater accumulation of cGMP in rat aortic rings when compared to PPDs (120). It was further elucidated that amongst all the known PPTs, Rg3 has the greatest vasodilatative effect (121). Rg3 has been found to induce endothelium-dependent relaxation in rat thoracic aorta via enhanced release of NO by upregulating the expression of nitric oxide synthase (122).

2.3.4.2 Clinical Evidence

Clinical data on the efficacy of KRG in improving blood pressure is limited. Nevertheless, daily intake of 4.5g KRG was observed to decrease 24 hour mean systolic blood pressure, compared with placebo, after eight weeks in 26 subjects with essential hypertension in a non-randomized, non-blinded crossover study (123).

Moreover, a randomized, controlled, double-blind, acute crossover study was conducted in healthy individuals in order to examine the effects of KRG root and its components (ginsenosides and polysaccharides) on peripheral BP and augmentation index (AI), a measure of arterial stiffness (124). Results of this study demonstrated a significant improvement in AI through the administration of both KRG root and an extract of its ginsenoside fraction three hours post-treatment, when compared to placebo (124). A similar acute study found a significant effect of an Rg3-enriched KRG extract on office blood pressure, central blood pressure and central AI three hours post-administration in healthy individuals (125). This demonstrated a potential role for Rg3 as a vasodilatory agent. In addition, an unpublished double-blind, randomized, controlled acute study conducted in hypertensive and pre-hypertensive individuals
revealed significant reductions in ambulatory systolic and diastolic blood pressure also following administration of an Rg3-enriched KRG extract.

2.3.5 Safety Profile of Ginseng

Although ginseng use has not been demonstrated to be toxic to humans, it has been associated with side effects, which must be considered in its safety profile (47). These include hypertension, nervousness, skin rash, insomnia, restlessness, anxiety, euphoria, vomiting, headache, nosebleed, breast pain, vaginal bleeding, diarrhea, confusion, depersonalization and depression (47)

However, in both acute and long-term studies conducted with AG and KRG at the Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada), ginseng use did not appear to compromise safety. Overall, studies revealed no differences between the ginseng-treated and the control groups in frequency and severity of adverse events, blood pressure and measures of renal, hepatic, and haemostatic function (10). The following outlines important considerations for ginseng safety in blood pressure and glycemia, and a systematic review of its safety in >100 clinical trials.

2.3.5.1 Blood Pressure

The potential hypertensive effect of ginseng has been a concern since the 1979 observational study conducted by Siegel revealed that its use may lead to elevated blood pressure (126). Siegel described a ‘Ginseng Abuse Syndrome’ where 22/133 patients presented with symptoms such as hypertension, euphoria, restlessness, nervousness, insomnia, skin eruptions, edema, and diarrhea
after long-term ginseng intake at variable doses (maximum dose of 15g) and variable routes of administration (126). Nevertheless, there was no control group in this study and participants used a variety of ginseng preparations including Siberian Ginseng, a species that does not contain ginsenosides (126). Moreover, blood pressure reportedly decreased in five subjects (126). However, based on this observational study, review papers have contraindicated ginseng in hypertension.

Conversely, clinical studies in the last decade have demonstrated a neutral or beneficial effect of ginseng use on BP. A 2006 study by Stavro et al examining the effects of AG use on blood pressure and renal function in hypertensive individuals found no significant effect on these parameters after 12 weeks administration of 3g/day AG (127). Nevertheless, two subjects reported experiencing headache and diarrhea (127). A similar study examining acute effects also demonstrated null effects of AG administration on BP (128).

### 2.3.5.2 Hypoglycemia

The insulin secretagogue effect demonstrated in clinical studies with AG suggests a potential for hypoglycemia. However, these studies also demonstrated that ginseng administration does not affect premeal glycemia and may instead act via glucose-stimulated insulin secretion, unlike conventional drug therapies, which act independent of a glucose stimulus (102,103,105).
2.3.5.3 A Summary of the Evidence

A 2002 systematic review of 146 clinical trials with ginseng reported that the incidence of adverse events in experimental groups taking ginseng was similar to that for placebo groups (129). The most frequently reported adverse events included headaches, sleep disturbances, and gastrointestinal effects. The review noted that the causality of adverse events based on isolated case reports submitted to national drug safety agencies is difficult to determine. Ginseng use was, for the most part, found to be well tolerated and its effects mild and reversible. Nevertheless, combination preparations, such as ginseng with another product, may be associated with some adverse effects, but the degree to which these are attributable to ginseng remains unclear (129). Potential herb-drug interactions of ginseng with phenelzine, warfarin and alcohol have been reported and therefore ginseng use for individuals using these products is cautioned (129). However, the World Health Organization (WHO) has proclaimed ginseng as without contraindications and serious adverse effects (130). The CDA, on the other hand, does not recommend the use of CAM in the treatment of diabetes at this time due to inadequate evidence for its safety and efficacy (1).

The therapeutic benefits of AG and KRG presented here, as well as the need for safety data warrant further investigation. Their combined treatment could simultaneously target two main abnormalities in T2DM and in doing so, provide a novel therapeutic option for diabetes management.
Chapter 3
Project Overview

3.1 Rationale

Clinical studies have demonstrated that AG may exert an insulin secretagogue effect much like the conventional therapy sulphonylurea; whereas KRG may act as an insulin sensitizer which is a mechanism of action of the conventional therapy metformin (9,42). This suggests that AG and KRG may complement one another in their mechanism of action, which may be exploited as a combination therapy.

Blood glucose levels can revert back to those at the time of diagnosis after approximately five years of treatment with monotherapy (14). Their secondary failure, that is, the loss of glycemic control after having initially been used as a successful treatment, necessitates the creation of novel treatment strategies (14,42,131). Combination therapy, or polypharmacy, is becoming more common to achieve tighter diabetes control (14). It has generally been demonstrated to be more effective than changing therapy or increasing the dosage of monotherapy -an approach that may lead to more pronounced side effects associated with that therapy (42,132).

Combination therapy takes advantage of medications with complementary mechanisms with the rationale that their complementary actions combined in therapy may act additively or synergistically (42). Several clinical trials of combination therapies have demonstrated additive HbA1c lowering efficacy of oral agents (14). A 1995 study revealed that the addition of metformin to sulphonylurea, the most frequently used combination, decreased HbA1c levels by an additional 1.7% after six months of treatment (133). Given the growing popularity of complementary and alternative medicine, an approach similar to combination therapy using alternative therapy may also prove to be an effective therapeutic option.
Since the National Health and Nutrition Examination Survey revealed that only 13% of patients with T2DM are able to concurrently meet target goals for glycemic control, lipids and blood pressure (6), and given the therapeutic potential of AG and KRG outlined in Chapter 2, further evaluation of parameters for each of these targets requires investigation.

3.2 Objective

The overall objective of this study was to examine the effect of 12 weeks administration of combined AG extract and KRG extract compared to placebo on the management of T2DM by examining measures of efficacy, safety and compliance.

The specific objectives are as follows:

Efficacy

Primary Objective: to evaluate the efficacy of combined AG and KRG on glycemic control through assessment of HbA1c.

Secondary Objectives: to evaluate the efficacy of combined AG and KRG on glycemic control [blood glucose, blood insulin] and other cardiovascular risk factors including blood lipids [low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG)], indices of blood pressure [office blood pressure, central blood pressure (BP), 24hr ambulatory BP, augmentation index (AI)] and low-grade body inflammation (hsCRP).
Safety

To monitor the effect of combined AG and KRG on safety measures including kidney function (creatinine), liver function (aspartate aminotransferase –AST), bleeding time (prothrombin time –PT, activated partial thromboplastin time –APTT, international normalized ratio –INR), and associated symptoms.

Compliance

To determine adherence to the study protocol by performing routine anthropometric measurements (height, weight, waist-to-hip ratio, body mass index, body composition), diet analysis (three-day food records) and a supplement count.

3.3 Hypothesis

Given the glycemia-lowering potential demonstrated in previous studies, a combined treatment of AG extract and KRG extract may improve glycemic control and other cardiovascular risk factors beyond that already attained through conventional treatment and have no adverse effects on safety parameters.

Specifically:

1. Combined use of AG and KRG, when compared to placebo, would improve glycemic control in well-treated patients, as indicated by reductions in HbA1c.

2. Combined use of AG and KRG, when compared to placebo, would also improve additional markers of glycemic control (blood glucose and insulin), lipid profile (LDL-C, TC, HDL-C, TG)
indices of blood pressure (office BP, central BP, 24hr ambulatory BP, augmentation index), and hsCRP in well-treated patients.

3. Combined use of AG and KRG will not affect safety parameters as indicated by kidney and liver function, bleeding time, and symptoms reporting.

3.4 Study Design

This study followed a placebo-controlled, randomized, double-blind two-arm parallel design in individuals with T2DM at two centres. The main centre was the Clinical Nutrition and Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada). This centre was responsible for all training, study documentation, testing materials and resources provided to the second centre. The second centre was located in a tertiary care facility, namely, the Division of Endocrinology, Diabetology, and Metabolic Disorders, Dubrava University Hospital (Zagreb, Croatia).
Chapter 4
Materials and Methods

4.1 Ginseng Preparation

A representative sample of AG obtained from major farms in Ontario was provided by the Ontario Ginseng Growers Association (Simcoe, Ontario, Canada), while KRG was provided by the National Agricultural Cooperative Federation (Chunk-buk, South Korea). All materials were tested for microbial contamination, heavy metal and pesticides, and fell within the predetermined acceptable ranges set by Health Canada (134).

AG roots aged three years or older were harvested and milled into a fine powder. Extracts were then prepared with 75% ethanol at room temperature. Liquid from the extraction was freeze-dried and milled into a fine powder. Similarly, Korean Ginseng roots aged four years or older were harvested and subsequently washed and steamed according to general production standards for KRG. Roots were then extracted with 95% ethanol at 70-75°C. Liquid from the extraction was dried and milled into a fine powder. Further detail about the preparation of the investigational products is considered proprietary by the manufacturer. The resulting ginseng extract was encapsulated into 500mg hard white opaque gelatin size 00 capsules.

4.1.1 Ginsenoside Analysis

The ginsenoside profile of both the AG and KRG extracts was analyzed using High Performance Liquid Chromatography with a diode array detector (DAD) and ultraviolet spectrophotometry (HPLC-DAD UV) courtesy of Dr. John Arnason at the University of Ottawa (Ottawa, Canada) as previously described in (135) and using methodology validated in (136). Extracts were first
prepared by adding 10mL of 70% methanol to 400mg of ground ginseng root and sonicating for 25 minutes at room temperature. The resulting solution was centrifuged and the supernatant removed. This was repeated twice using 10mL and then 4mL of 70% methanol. A mixture of 1mL of the extract and 100μL 5% KOH was then incubated for two hours in the dark, subsequently neutralized with 14% KH$_2$PO$_4$, and then filtered through a 0.2μm polytetrafluoroethylene filter (Chromatographic Specialties Inc., Brockville, Ontario, Canada).

Solvents used for analysis were HPLC-grade and purchased from Fisher Scientific (Ottawa, Ontario, Canada). Ginsenoside standards for Rg1, Re, Rb1, Rb2, Rc, and Rd were purchased from Indofine Chemical Company Inc. (Hillsborough, New Jersey, USA) and the standard for Rg3 was purchased from LKT Laboratories Inc (St. Paul, Minnesota, USA). A standard mix of pure ginsenosides was also provided by Dr. Anthony Windust (NRC, Ottawa, Ontario, Canada). The Hewlett-Packard Chemstation series 1100 chromatograph (Agilent, Palo Alto, California, USA) with a DAD and Phenomenex Luna C-18 column (150mmx4.6mm; 5μm particle size; 100A pore size) was used with temperature and flow rate controlled at 25°C and 1.5 mL/min, respectively. HPLC-grade water and acetonitrile were used as the mobile phase. UV detection was set at 203 nm.

Elution profiles of ginsenosides in the investigated samples were compared with PPD (Rb1, Rb2, Rc, Rd, Rg3) and PPT (Re, Rg1) standards and quantified. Species identification was confirmed on the basis of the presence of Rg3 in KRG, and the Rb1/Rg1, Rg1/Re, and Rb2/Rc ratios in AG.

The ginsenoside profile (major ginsenosides, total PPD, PPT, ginsenoside content and PPD/PPT ratio) of the AG and KRG extracts provided to subjects is presented in Table 4-1.
Table 4-1. Ginsenoside profiles for AG and KRG extract presented as mean of triplicate analysis±SD.

<table>
<thead>
<tr>
<th>Species</th>
<th>AG</th>
<th>KRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginsenoside</td>
<td>% (w/w)</td>
<td></td>
</tr>
<tr>
<td>Rg1</td>
<td>0.085±0.002</td>
<td>0.170±0.002</td>
</tr>
<tr>
<td>Re</td>
<td>0.8953±0.0004</td>
<td>0.3030±0.0002</td>
</tr>
<tr>
<td>Total PPT</td>
<td>0.982±0.002</td>
<td>0.473±0.002</td>
</tr>
<tr>
<td>Rb1</td>
<td>3.2802±0.0006</td>
<td>1.89±0.02</td>
</tr>
<tr>
<td>Re</td>
<td>0.2993±0.0007</td>
<td>1.01±0.03</td>
</tr>
<tr>
<td>Rb2</td>
<td>0.048±0.001</td>
<td>0.77±0.01</td>
</tr>
<tr>
<td>Rd</td>
<td>0.429±0.008</td>
<td>0.47±0.06</td>
</tr>
<tr>
<td>Total PPD</td>
<td>4.064±0.008</td>
<td>4.15±0.07</td>
</tr>
<tr>
<td>Total</td>
<td>5.046±0.008</td>
<td>4.62±0.07</td>
</tr>
<tr>
<td>PPD:PPT</td>
<td>4.137±0.008</td>
<td>8.77±0.07</td>
</tr>
</tbody>
</table>
4.2 Wheat Bran Preparation

Red hard wheat bran was purchased from the American Association of Cereal Chemists International (St. Paul, Minnesota, USA) and encapsulated into 500mg hard white opaque gelatin size 00 capsules.

4.3 Recruitment and Subject Selection

4.3.1 Power Analysis

Given the parallel design of the study and previous observations of studies performed in T2DM, a treatment difference of 0.8% in HbA1c (SD=1.0%) was used for determination of sample size using a T-test. Analysis indicated that approximately 25 individuals per group would be required given $\alpha=0.05$ and $1-\beta=0.8$. Assuming an attrition rate of 35%, a total of 80 subjects were to be recruited.

4.3.2 Recruitment and Screening

Individuals were recruited by contacting past volunteers at the Risk Factor Modification Centre, St. Michael’s Hospital, and through the use of poster advertisements and newspaper advertisements. Recruitment was carried out in two phases. Phase I was conducted in the first quarter of 2009 and relied on the RFMC patient database and poster advertising, while phase II was conducted in the final quarter of 2009 and relied on newspaper advertising. Phase II also marked the beginning of the study at the second centre where subjects were recruited using a roster of their regular patients: advertising was not required.
Interested volunteers were initially screened by telephone questionnaire (Appendix 1). Groups of one to four individuals were invited to attend an information session during which the exact nature of the study was described and individuals were given as much time as they felt necessary to have all questions answered. Individuals were provided a copy of the consent form to take home with them and instructed to contact the clinic if they were still interested to schedule the screening visit. For the screening assessment, potential subjects attended the clinic after a 10-12 hour overnight fast. The study was explained again and any remaining questions about the study addressed. Those willing to join the study were asked to sign the consent form (Appendix 2). A fasting blood sample was then drawn, anthropometric measurements collected and blood pressure taken. Subjects completed a detailed questionnaire concerning their medical history, drugs and medication use (including ginseng, herbs, vitamin and minerals), smoking habits, alcohol intake, exercise pattern and diet (Appendix 3). Subjects were advised to stay on their current treatment medication regimen as prescribed by their family doctor since study treatment would serve as an adjunct to their current medication. They were asked to report any change in medical status and drugs as well as use of natural health products at run-in and at each treatment visit. They were also asked to maintain a constant level of physical activity, maintain a constant diet and adhere to their usual lifestyle during the course of the study.

4.3.3 Subject Selection and Randomization

Eligibility was determined using the participation criteria described hereafter.

*Inclusion Criteria:* Individuals with T2DM for at least 1 year and treatment with diet and/or oral hypoglycemic medication that was unchanged starting at least two months prior to the study;
between the ages of 30 and 75 years; systolic blood pressure <140 and diastolic blood pressure <90; clinically euthyroid; normal renal and hepatic functions; willing to comply with the study protocol and sign a consent form; women that were either post-menopausal (more than a year of cessation of menstruation), or non-pregnant (pregnancy test administered at screening).

**Exclusion Criteria:** Individuals taking insulin; with bleeding disorders, planned surgery, pregnancy or breastfeeding; clinically significant diabetes complications (retinopathy, nephropathy, or neuropathy); serum triglycerides ≥ 4.5 mmol/L; history of angina or heart attack; use of ginseng within two months; BMI >35 kg/m² and/or a weight fluctuation of ±2kg during the treatment periods; smoke cigarettes; alcohol intake greater than two drinks/day; 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, if the subject participated in the study; subjects taking Warfarin, Coumadin, prescription non-steroidal anti-inflammatory drugs (NSAIDs), chronic use of high-dose (>81mg) non-prescription NSAIDs, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors, sympathomimetics, or individuals with any known or suspected sensitivity to any of the ingredients in the test product or placebo could not participate in the study.

The investigational products were approved by the Natural Health Products Directorate of Health Canada (Appendix 4) and the study by the St. Michael’s Hospital Research Ethics Board (Appendix 5) and the Dubrava University Hospital Ethics Board. Approval was also obtained from the University of Toronto Research Ethics Office (Appendix 6). Clinical follow-up was carried out at the Risk Factor Modification Centre, St. Michael’s Hospital (Toronto, Canada) and the Division of Endocrinology, Diabetology and Metabolic Disorders, Dubrava University Hospital (Zagreb, Croatia).
Randomization to treatment was done using a computer-generated random number table. Subjects were assigned to consecutive numbers after they provided written informed consent.

4.4 Intervention

Subjects were provided with capsules at every follow-up visit and instructed to consume two 500mg capsules prior to each meal for a total of six capsules each day (3g/day). This dose was similar to the long-term studies belonging to the acute-to-chronic clinical testing programs for AG and KRG, and based on the findings of the acute studies previously conducted at the RFMC (10,102,104,105,108,109). These programs found that the minimum doses tested, 1g of AG and 2g KRG rootlets, were effective preparations of ginseng in their ability to improve glycemia (10,102,104,105,108,109).

Consumption of treatment capsules was advised as an adjunct to current medications, diet and lifestyle. Subjects were provided with capsules in a quantity sufficient to last them an additional three to seven days beyond the next visit. Capsules were matched for macronutrient content and appearance for both study arms. All treatments were coded by an individual otherwise not involved in the study in order to ensure the blinding of study personnel and subjects.

4.5 Study Protocol and Timeline

Eligible subjects as assessed by the participation criteria were invited to attend the clinic to start the four week open-label run-in period during which they were provided placebo capsules to
habituate them to taking the supplement and stabilize baseline measures. Subsequently, subjects began the 12 week treatment phase of the study.

During the treatment phase, subjects attended the clinic for follow-up visits at regular intervals for examination as outlined in Figure 4-1. Subjects completed a clinical assessment (Appendix 7) and a symptoms questionnaire (Appendix 8) at every visit. They were also asked to return any remaining capsules at each visit so that compliance could be assessed. A new supply of the supplement was provided at that time.

**Figure 4-1.** Study timeline. Subjects attended the clinic for an information session, screening visit and then several follow-up visits for four months.
4.6 Study Measurements

**Table 4-2.** Protocol of measurements conducted at each visit, relative to treatment initiation (week 0).

<table>
<thead>
<tr>
<th>MEASUREMENT</th>
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<th>3</th>
<th>6</th>
<th>12</th>
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<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
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<td>Anthropometric Measurements</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
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<td>Office Blood Pressure</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Capsule Replenishment</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3hr OGTT</td>
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<td></td>
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<tr>
<td>Three Day Food Record</td>
<td>X</td>
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<td>X</td>
<td></td>
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<td>Quality of Life Questionnaire</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Symptoms Questionnaire</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Augmentation Index</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Central Blood Pressure</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24hr Ambulatory Blood Pressure</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

OGTT- oral glucose tolerance test
4.6.1 Blood Samples

Blood samples were taken after a 10-12 hour overnight fast at the screening visit and baseline, middle and end of treatment. A phlebotomist withdrew blood from the forearm in SST, EDTA or citrate-treated vacutainer tubes (BD Diagnostics, Quebec, Canada). All blood samples obtained from the forearm were analyzed by the Core Lab, St. Michael’s Hospital, Toronto using standard laboratory methodology (Table 4-3).

4.7 Analytical Procedures

The following description of analytical methods pertains to analysis conducted at centre one. This information was not available for centre two.

4.7.1 Glycemic Parameters

4.7.1.1 Biochemical Parameters

Whole blood analysis of HbA1c was performed using high performance liquid chromatography with the Tosoh HLC-723 analyzer. A cation exchange column and gradient salt elution were used to separate HbA1c from HbA. HbA1c was expressed as a fraction of the total hemoglobin in the sample (137).

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 (138).
Table 4-3. Biochemical parameters evaluated following a fasting blood test at St. Michael’s Hospital.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>MEASUREMENT</th>
<th>BLOOD ANALYSIS</th>
<th>INSTRUMENT</th>
<th>TECHNIQUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycemic Control</td>
<td>Whole Blood</td>
<td>Tosoh Bioscience HLC-723</td>
<td>HPLC</td>
</tr>
<tr>
<td>FBG</td>
<td>Glycemic Control</td>
<td>Serum</td>
<td>Beckman Coulter Synchron</td>
<td>Rate of Oxygen Consumption</td>
</tr>
<tr>
<td>FBI</td>
<td>Glycemic Control</td>
<td>Serum</td>
<td>Beckman Coulter UniCel Dxl 600 Access</td>
<td>Immunoenzymatic</td>
</tr>
<tr>
<td>hsCRP</td>
<td>Low-grade Body Inflammation</td>
<td>Serum</td>
<td>Beckman Coulter Synchron</td>
<td>Turbidimetric</td>
</tr>
<tr>
<td>LDL-C, TC, HDL-C, TG</td>
<td>Lipids</td>
<td>Serum</td>
<td>Beckman Coulter Synchron</td>
<td>Oxidase, LDL-C=TC-(HDL-C+TG/2.2)</td>
</tr>
<tr>
<td>Safety</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST, Cr</td>
<td>Liver and Kidney Function</td>
<td>Serum</td>
<td>Beckman Coulter Synchron</td>
<td>Spectrophotometric</td>
</tr>
<tr>
<td>PT, INR, APTT</td>
<td>Bleeding Time</td>
<td>Plasma</td>
<td>Instrumental Laboratory ACL TOP</td>
<td>Time to Clot Formation</td>
</tr>
</tbody>
</table>

HbA1c- glycated hemoglobin; HPLC- high performance liquid chromatography; FBG- fasting blood glucose; FBI- fasting blood insulin; hsCRP- high-sensitivity C-reactive protein; TC- total cholesterol; HDL-C- high-density lipoprotein cholesterol; LDL-C- low-density lipoprotein cholesterol; TG- triglycerides; AST- aspartate aminotransferase; Cr- creatinine; PT- prothrombin time; INR- international normalized ratio; APTT- activated partial thromboplastin time
Serum insulin was analyzed using immunoenzymatics with the Beckman Access Ultrasensitive Insulin Assay (Beckman Coulter, Brea, CA). Insulin was separated from the sample using immunoprecipitation with magnetic particles, and subsequently reacted with a chemiluminescent substrate to generate light (139). 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 (139).

4.7.1.2 Oral Glucose Tolerance Test

A finger prick capillary blood sample was taken at baseline (time 0) following which a 300mL orange flavoured 75g glucose drink (GLUCODEX® Ratiopharm Inc, Mississauga, Canada, DIN:00509965) was administered with two 500 mg capsules over a period of five minutes. Additional blood samples were drawn at 30, 60, 90, 120 and 180 minutes after the start of the test drink.

Whole blood samples (25-75uL) were collected in 7mL fluoride oxalate-treated flat base polystyrene tubes (Sarstedt Inc, Montreal, Canada) and immediately stored at -20ºC pending analysis. Tubes were prepared with 375µg sodium fluoride and 300ug potassium oxalate and allowed to air dry for three days prior to use. Whole blood samples were analyzed for glucose concentration using the YSI 2300D STAT Plus Glucose & Lactate Analyzer (Yellow Springs, Ohio, USA) within three days of collection. The analytical method employed by the YSI is based on the oxidation of glucose leading to electron production and the subsequent generation of an electric current that is linearly proportional to the glucose concentration (140). The YSI was
calibrated with a standard 10mmol/L glucose solution prior to and during analysis of each set of seven samples.

4.7.2 Lipid Parameters

The Beckman SYNCHRON LX System was used to analyze serum total/fasting cholesterol (TC). This method determined cholesterol concentration by a timed-endpoint method. Cholesterol esterase was used to hydrolyze cholesterol esters in the sample to free cholesterol and fatty acids (138). Cholesterol oxidase then oxidized free cholesterol leading to the formation of hydrogen peroxide which subsequently reacted to produce a colored quinoneimine product (138). The change in absorbance, measured at 520nm, was directly proportional to the concentration of cholesterol in the sample (138).

The Beckman SYNCHRON LX System was used to determine the concentration of serum triglycerides (TG) by a timed-endpoint method. Lipase was used to hydrolyze TG in the sample to glycerol and free fatty acids (138). Three sequential enzymatic reactions with glycerolkinase, glycerophosphate oxidase and horseradish peroxidase then lead to the formation of a red quinoneimine dye (138). The change in absorbance, measured at 520nm, was directly proportional to the concentration of TG in the sample (138).

High-density lipoprotein cholesterol (HDL-C) was measured using the Beckman SYNCHRON LX Systems. HDL-C in the sample was first solubilized from HDL lipoprotein particles and then reacted with cholesterol esterase and cholesterol oxidase to generate hydrogen peroxide that in the presence of chromogens, produced a color product (138). The same detergent used for solubilization, also inhibited the reaction of cholesterol enzymes with low density, very-low
density, and chylomicron lipoproteins (138). The reagent contained a polyanion that complexed low-density, very low-density and chylomicron lipoproteins and in doing so, improved the selectivity for HDL-C (138). The change in absorbance, measured at 560nm, was directly proportional to the concentration of HDL-C in the sample (138).

Serum low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula:

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

(141,142)

This equation is only valid when triglycerides are present at a concentration \(<4.52 \text{ mmol/L} \) (142). LDL-C, therefore, could not be calculated for \( \text{TG} \geq 4.52 \text{ mmol/L} \)

### 4.7.3 Low-Grade Body Inflammation

Serum high-sensitivity C-Reactive Protein (hsCRP) was analyzed using the Beckman SYNCHRON LX System via turbidimetry. HsCRP in combination with a specific antibody formed an insoluble antigen-antibody complex (138). The change in absorbance resulting from the formation of this complex, measured at 340nm, was proportional to the concentration of hsCRP in the sample (138).

### 4.7.4 Parameters of the Blood Pressure Waveform

#### 4.7.4.1 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). Subjects
remained seated in a quiet, temperature-controlled room for 5-10 minutes with arm supported at heart level to achieve resting heart rate and blood pressure. Subsequently, three readings were obtained from the brachial artery in the left forearm, with one minute separating each measurement. The arithmetic mean of the three readings was used in all analysis.

4.7.4.2 Arterial Stiffness and Central Blood Pressure

Subjects underwent a non-invasive measurement of arterial stiffness using applanation tonometry (SphygmoCor, AtCor Medical, West Ryde, Australia) at baseline, middle and end of treatment. A tonometer was used as a pressure sensor at the radial artery to obtain a measure of Augmentation Index (AI) and central blood pressure via Pulse Wave Analysis using the software’s validated algorithm. Subjects were required to lie down in a supine position to minimize movement.

4.7.4.3 Ambulatory Blood Pressure

24 hour ambulatory blood pressure (AMBP) was monitored oscillometrically at the beginning and end of the treatment phase using the Spacelabs 90207 system (Mississauga, Ontario, Canada). The cuff was secured on the nondominant arm and measurements taken every half hour from 7:00am to 11:00pm and every hour from 11:00pm to 7:00am. Measurements were automatically repeated after two minutes if an error occurred or if obtained readings fell outside predefined acceptable ranges.
4.7.5 Safety Measures

4.7.5.1 Biochemical Parameters

Serum aspartate aminotransferase (AST) activity was 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+ (138). The rate of change in absorbance, measured at 340nm, was directly proportional to the activity of AST (138).

The SYNCHRON LX System was used to determine serum creatinine concentration by the Jaffe rate method. Creatinine reacted with a reagent to produce a red color 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 creatinine in the sample (138).

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 (143). Factor XII was activated using a phospholipid reagent composed of lipids and an activator reagent (143).

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 (144). This resulted in the conversion of fibrinogen to fibrin and subsequent formation of a solid gel (144). The time required for clot formation was measured as PT (144).
The International Normalized Ratio (INR) was calculated from PT and mean PT normal range of a control sample according to the formula:

\[
INR = \frac{PT_{\text{test}}^{\text{SI}}}{PT_{\text{normal}}} \quad (144)
\]

where ISI is the International Sensitivity Index based on the tissue factor used to activate the reaction (144).

4.7.5.2 Questionnaires

A medical information form was administered at the screening visit. This form collected data on medical history, current medication use and daily exercise/diet regimens.

Owing to the few references in literature reporting on possible adverse effects of ginseng, including headache, insomnia, and nervousness, a questionnaire including questions related to adverse effects was administered at every visit.

4.7.6 Compliance

4.7.6.1 Supplement Consumption

Supplement compliance was assessed by counting returned capsules. Compliance was calculated using the following formula:
Compliance

\[ \text{Compliance} = 100 \left( \frac{\# \text{ capsules consumed for } x \text{ days}}{\# \text{ capsules prescribed for } x \text{ days}} \right) \]

where \( x \) was the days of treatment (approximately 84).

4.7.6.2 Anthropometric Assessment

At every visit, subjects underwent anthropometric measurements including height, weight, assessment of waist-to-hip ratio, and body composition.

Height was measured with a wall-mounted stadiometer (Perspective Enterprises, Portage, MI) with head in the “Frankfurt horizontal” position in barefooted subjects and rounded to the nearest centimeter. Waist circumference was measured using a non-stretchable measuring tape positioned at the level of noticeable waist narrowing and recorded to the nearest centimeter. Similarly, the hip girth was measured with the tape measure positioned at the level of the widest point of the hip and recorded to the nearest centimeter. After emptying of bladder, removing any excess clothing and shoes, weight, and body composition (BMI, percent body fat) were analyzed via Bioelectrical Impedance Analysis using the TANITA BC-418 Segmental Body Composition Analyzer (Arlington Heights, Illinois, USA).

4.7.6.3 Diet Analysis

Three day diet records (Appendix 9) were analyzed using ESHA Food Processor SQL, version 9.8 (Salem, Oregon, USA).Subjects completed a three day diet record at the beginning of the
run-in period to receive training on how to properly complete the record. An average of the three day profile was generated for the records obtained at the beginning and end of the treatment phase of the study only. Diet was analyzed for total caloric intake and macronutrient intake.

4.7.7 Statistical Analysis

Statistical analyses were performed using the Number Cruncher Statistical System (NCSS) 2000 software (NCSS statistical software, Kaysville, Utah). End values in all efficacy parameters were assessed using multivariate multiple regression. Where data was not obtained from the second centre at the time of data analysis and interpretation, it was not included, and is indicated accordingly. All data was adjusted for baseline values, BMI, centre (where applicable) and tested for normality using the Shapiro-Wilk test. Data not following a normal distribution was presented as median (interquartile range) and tested for differences using the Mann-Whitney U-test for non-parametric data. Subject characteristics were expressed as mean±standard deviation (SD) while all other data was presented as mean±standard error of the mean (SEM). Baseline differences in subject characteristics were evaluated using Student’s T-test and Chi-Square/Fisher’s Exact test.

Comparison of differences from baseline to treatment-end in all parameters of safety and compliance were assessed within treatment arms using a Student’s T-test. Data was considered statistically significant at p<0.05.
Chapter 5
Results

5.1 Study Participants

Sixty-four subjects with T2DM from two centres were enrolled in this study ($n_{\text{centre one}}=33$, $n_{\text{centre two}}=31$). From the 191 individuals who contacted centre one (Figure 5-1) wishing to participate, 171 were telephone screened. From the individuals that were telephone screened, 93 attended an information session, 46 of whom subsequently underwent a clinical screening visit. Of the 33 subjects that met the eligibility criteria and were enrolled, six subjects were lost to follow-up; three did not meet the eligibility criteria, two were unable to make the time commitment and one withdrew due to undesired side effects. In addition, one subject had not yet completed the study at the time data analysis took place. At centre two, one subject was excluded due to inadequate supplement consumption ($<70\%$) and eight subjects had not yet completed the study at the time of data analysis. A detailed flow of subjects at centre two was not available.
Figure 5-1. Subject flow from initial contact until study completion for centre one.
The results presented herein are therefore for forty-eight subjects with a distribution between treatment arms and centres as shown in Table 5-1.

**Table 5-1.** Completed subjects by treatment arm and centre.

<table>
<thead>
<tr>
<th>Centre</th>
<th>PLACEBO</th>
<th>AG+KRG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centre One (n)</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Centre Two (n)</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Total (n)</td>
<td>22</td>
<td>26</td>
</tr>
</tbody>
</table>

Baseline subject characteristics shown in Table 5-2 present an average of all baseline visits where possible. This includes screening, the beginning of the run-in period (week -4) and week 0. HbA1c and fasting blood glucose (FBG) results are presented for the week 0 visit only.

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 also comparable; one subject in the placebo arm was not taking any medication, and this was also true for three subjects in the ginseng arm. The number of subjects taking mono- as compared to combination oral anti-hyperglycemic therapy was evenly distributed in both study groups (i.e. 9/20 subjects were taking combination therapy in the placebo arm compared to 10/20 in the ginseng arm). Oral anti-hyperglycemic medications included biguanides (Metformin: Glucophage, Glumetza, Siofor, Gluforin), sulphonylureas (Glyburide, Amaryl, Diaprel, Diaprel MR, Diglical, Meglimid, Glurenorm, Gliklada), thiazolidinediones (Rosiglitazone, Pioglitazone), α-glucosidase inhibitors (Acarbose, Glucobay) and combinations of these (Avandamet). Antihypertensive medications included angiotensin-converting enzyme inhibitors (Ramipril, RAN-Lisinopril, Mavik, Vasotec, Tritace, Piramil, Irumed, Prexanil, Prinivil,
Table 5-2. Between-group differences at baseline presented as mean±SD for anthropometric and demographic data and mean±SEM for clinical data.

<table>
<thead>
<tr>
<th>SUBJECT CHARACTERISTIC</th>
<th>TREATMENT</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PLACEBO</td>
<td>AG+KRG</td>
</tr>
<tr>
<td>Sample Size (n)</td>
<td>22</td>
<td>26</td>
</tr>
<tr>
<td>Sex (n)</td>
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<td>Male</td>
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<td>15</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62±9</td>
<td>59±10</td>
</tr>
<tr>
<td>Duration of Diabetes (years)</td>
<td>5 (3,8)</td>
<td>3 (2,10)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5±4.0</td>
<td>31.5±5.7</td>
</tr>
<tr>
<td>BF (%)</td>
<td>32±9</td>
<td>34±7</td>
</tr>
<tr>
<td>WHR (male)</td>
<td>0.96 ± 0.04</td>
<td>0.97 ± 0.05</td>
</tr>
<tr>
<td>WHR (female)</td>
<td>0.91 ± 0.09</td>
<td>0.89 ± 0.05</td>
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<tr>
<td>Systolic BP (mm Hg)</td>
<td>137±5</td>
<td>141±5</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>78±3</td>
<td>81±2</td>
</tr>
<tr>
<td>Medication Use (n)</td>
<td>T2DM</td>
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<tr>
<td></td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>CVD</td>
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<td>15</td>
</tr>
<tr>
<td>BP</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.2 ± 0.2</td>
<td>7.2 ± 0.2</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>7.1 (6.1,7.9)</td>
<td>6.8 (5.9,8.9)</td>
</tr>
</tbody>
</table>

AG- American Ginseng; KRG- Korean Red Ginseng; BMI- body mass index; BF- body fat; WHR- waist-to-hip ratio; BP- blood pressure; T2DM- type 2 diabetes mellitus; CVD- cardiovascular disease; HbA1c- glycated hemoglobin; FBG- fasting blood glucose
Monopril, Cilazil, Enap, Gopten), angiotensin II receptor antagonists (Losartic, Aprovel, Co-Aprovel, Cozaar, Diovan), calcium channel blockers (Diltiazem, Adalat, Amlopin, Lacipil, Lercanil, Lopin), diuretics (Novo-Hydrazide, Sanofi-Aventis, Hygroton), direct renin inhibitors (Rasilez), β-Blockers (ratio-Atenolol, Bisoprolol, Nibel), α-adrenergic receptor agonists (Apo-Methyldopa), central alpha adrenergic agonists (Physiotens) and combinations of these (Hyzaar, Tritazide, Iruzid, Prexanil Combi, Avalide). Cholesterol medications included statins (Crestor, Lipitor, Zocor, Tulip, Sortis, Lipex, Sortis, Atorvox, Atoris, Simvax), nicotinic acid (Niaspan), fibrates (TriCor) and combinations (Inegy).

Anthropometric measures of body fat (BF) and waist-to-hip ratio (WHR) were also similar between the groups, however, baseline body mass index (BMI) was significantly higher (p=0.047) in the group randomized to ginseng treatment. All data was therefore adjusted for baseline BMI.

**Between-Centre Differences**

The differences in the sample population examined at each centre were also considered in statistical analysis. Analysis of baseline differences, as shown in Table 5-3, revealed that subjects at the second centre were generally heavier and more hypertensive than subjects at centre one. Accordingly, more subjects at centre two were taking hypotensive medications.
Table 5-3. Between-centre differences at baseline presented as mean±SD for anthropometric and demographic data and mean±SEM for clinical data.

<table>
<thead>
<tr>
<th>SUBJECT CHARACTERISTIC</th>
<th>TREATMENT</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CENTRE ONE</td>
<td>CENTRE TWO</td>
</tr>
<tr>
<td>Sample Size (n)</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Sex (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59±9</td>
<td>63±10</td>
</tr>
<tr>
<td>Duration of Diabetes (years)</td>
<td>4 (2.11)</td>
<td>4 (2.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (25.2,31.1)</td>
<td>32.0 (30.1,35.4)</td>
</tr>
<tr>
<td>BF (%)</td>
<td>30±8</td>
<td>37±6</td>
</tr>
<tr>
<td>WHR (male)</td>
<td>0.95 ± 0.05</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>WHR (female)</td>
<td>0.87 ± 0.08</td>
<td>0.93 ± 0.06</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>125±3</td>
<td>156±5</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>74±2</td>
<td>86±2</td>
</tr>
<tr>
<td>Medication Use (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2DM</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>CVD</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>BP</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.8 (6.4,7.6)</td>
<td>7.4 (6.8,7.7)</td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
<td>6.8±0.3</td>
<td>7.7±0.4</td>
</tr>
</tbody>
</table>

BMI- body mass index; BF- body fat; WHR- waist-to-hip ratio; BP- blood pressure; T2DM- type 2 diabetes mellitus; CVD- cardiovascular disease; HbA1c- glycated hemoglobin; FBG- fasting blood glucose
5.2 Treatment of Missing Data

The three hour OGTT was conducted only at centre one where all subjects participated with the exception of two who could not make the time commitment. Similarly, central blood pressure, central augmentation index and ambulatory blood pressure were evaluated only at centre one. One subject at centre one discontinued their diabetes medication half-way into the treatment phase of the study, their glycemic data was therefore excluded from analysis. A different subject at centre one began taking cholesterol medication three weeks into the treatment phase of the study, their lipidemic data was therefore excluded from analysis.

Another subject stopped taking Hyperexol, a supplement marketed as an antihypertensive, half-way through the treatment phase. This subject was in the ginseng arm of the study and their blood pressure data was included in analysis owing to the unknown efficacy of this product. If the product is effective, termination of its use would negatively affect BP thus potentially reducing the effect size of ginseng use.

One subject at centre one did not wish to partake in the ambulatory blood pressure monitoring. They are therefore missing these data. Moreover, six subjects did not undergo nocturnal monitoring (n=3 in each arm). Accordingly, their 24 hour means were also excluded because they are not representative of a full day but instead only diurnal averages.

Four subjects were missing LDL-C values. These could not be calculated with the algorithm used owing to TG levels greater than 4.52 mmol/L.

Missing data values for reasons including failure to analyze blood samples for all indicated tests by the Core Lab, insufficient capillary blood samples due to poor bleeding during the OGTT, and equipment failures were calculated. Missing values at baseline or end of treatment were recorded
as intermediate values. This treatment of missing values minimized the potential effect size and in doing so, favoured the null hypothesis. Missing values during the intermediate visit were calculated using an average of baseline and end of treatment values. Nevertheless, intermediate values were generally irrelevant in the analysis presented here since end-point differences were evaluated. Intermediate values did however play a role in generating the graphs shown.

5.3 Efficacy of Combined AG and KRG on Glycemia

5.3.1 Glycated Hemoglobin

Data for glycated hemoglobin was not normally distributed and a logarithmic transformation did not amend this distribution. Therefore, end values in group medians were compared using the Mann-Whitney U-test to test for differences in medians. For individuals in the placebo arm (n=22), HbA1c [median (IQR)] increased from 7.1 (6.5,7.8) % at baseline to 7.3 (6.7,8.1) % at treatment-end, while it decreased from 7.1 (6.4,7.6) % at baseline to 6.6 (6.4,7.7) % at treatment-end in the ginseng arm (n=25) (Figure 5-2). However, analysis failed to demonstrate a significant difference between the two treatment arms at treatment-end (p=0.1) or from baseline to treatment-end within either group.
Figure 5-2. The effect of combined AG and KRG (n=25) compared to placebo (n=22) on HbA1c at baseline, middle and end of treatment, n=45. End values were compared using the Mann-Whitney U-test. Results are presented as medians with the interquartile range indicated by the vertical lines.
5.3.2 Effect on Secondary Outcome Measures

5.3.2.1 Fasting Blood Glucose and Insulin

Analysis of logarithmically-transformed fasting serum glucose and fasting serum insulin data revealed that there was no significant effect of the combined use of AG and KRG on end values when compared to the control arm (Figure 5-3 and Figure 5-4).

5.3.2.2 Postprandial Glycemia

Analysis of the glycemic response to a 75g oral glucose load revealed no significant effect of treatment at 0, 30, 60, 90, 120 and 180 minutes of the glucose tolerance test both between and within treatment groups from baseline to treatment-end (Figure 5-5). Data was adjusted for time zero and BMI. Similarly, analysis of incremental area under the glucose curve showed that there was no significant improvement from baseline to treatment-end within treatment groups, and no effect of treatment when examining end values of the two treatment groups (data not shown here).

5.4 Efficacy of Combined AG and KRG on Lipid Parameters

Analysis of end values in both study arms revealed that there were no significant improvements in logarithmically-transformed serum TC levels, HDL-C and TG associated with combination ginseng use. LDL-C was also not significantly affected (Table 5-4).
Figure 5-3. The effect of combined AG and KRG (n=25) compared to placebo (n=22) on fasting serum glucose levels at baseline, middle and end of treatment, n=47. Results are presented as unadjusted mean±SEM.
**Figure 5-4.** The effect of combined AG and KRG (n=25) compared to placebo (n=22) on fasting serum insulin levels at baseline, middle and end of treatment. Results are presented as unadjusted mean±SEM.
Figure 5-5. Effect of combined AG and KRG (n=12) compared to placebo (n=12) on postprandial glycemia assessed at baseline and end of treatment, n=24. Results are presented as unadjusted mean±SEM.
Table 5-4. Comparison of end values in lipidemic parameters. Results are presented as unadjusted mean±SEM or median (IQR) for non-normal data. For all parameters n=47, except LDL cholesterol for which n=43.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO (n=21)</th>
<th>AG+KRG (n=26)</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>4.6 (3.7, 5.2)</td>
<td>5.2 (4.0, 5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.1 (1.0, 1.3)</td>
<td>1.2 (1.0, 1.3)</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>2.6 ± 0.2 (n=19)</td>
<td>2.7 ± 0.2 (n=24)</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.7 (1.0, 2.9)</td>
<td>1.7 (1.2, 2.8)</td>
<td>NS</td>
</tr>
</tbody>
</table>

TC- total cholesterol; HDL-C- high-density lipoprotein cholesterol; LDL-C- low-density lipoprotein cholesterol; TG- triglycerides
5.5 Efficacy of Combined AG and KRG on Low-Grade Body Inflammation

Data for two-centre low-grade body inflammation (hsCRP) was lacking a normal distribution. Since the distribution was not rectified following data transformation, non-parametric testing was used for analysis. The Mann-Whitney U-test was used to test for differences in medians comparing baseline values to end values within each group. For individuals in the placebo arm (n=20), median hsCRP [median (IQR)] was 1.4 (0.7,2.3) mg/L at baseline and 1.2 (0.4,2.2) mg/L after 12 weeks. For individuals in the combined AG and KRG arm (n=21), median hsCRP was 1.9 (0.6,3.8) mg/L at baseline and 1.7 (0.9,3.2) mg/L after 12 weeks. There were no significant differences from baseline to treatment-end within either group.

5.6 Efficacy of Combined AG and KRG on Blood Pressure and Augmentation Index

5.6.1 Office Blood Pressure

The combined use of AG and KRG demonstrated a trend toward lower brachial systolic blood pressure (SBP) compared to the use of placebo after 12 weeks administration in 48 individuals with T2DM. However, these results were only borderline significant with p=0.052. Data was adjusted for baseline values, BMI, and centre. The potential effect size of combined AG and KRG ($\beta$ [95% Confidence Interval]) was found to be -8.3 [-16.6, 0.06] mm Hg.

Brachial DBP and logarithmically-transformed pulse pressure (PP) were not found to be significantly affected by treatment (Table 5-5).
Figure 5-6. The effect of combined AG and KRG (n=26) compared to placebo (n=22) on brachial systolic blood pressure at baseline, middle and end of treatment, n=48. Results are presented as unadjusted mean±SEM.
Table 5-5. The effect of combined AG and KRG (n=26) compared to placebo (n=22) on brachial diastolic and pulse pressure, n=48. Results are presented as unadjusted mean±SEM.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO</th>
<th>AG+KRG</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBP (mm Hg)</td>
<td>79 ± 3</td>
<td>78 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>PP (mm Hg)</td>
<td>60 ± 5</td>
<td>56 ± 3</td>
<td>NS</td>
</tr>
</tbody>
</table>

DBP- diastolic blood pressure; PP- pulse pressure

5.6.2 Arterial Stiffness

5.6.2.1 Peripheral Augmentation Index

Data for two-centre peripheral AI was lacking a normal distribution. Since the distribution was not rectified following data transformation, the Mann-Whitney U-test was used to test for differences in medians comparing baseline values to end values within each group. For individuals in the placebo arm (n=22), median peripheral AI [median (IQR)] was 88 (82,93) % at baseline and 86 (82,91) % after 12 weeks. For individuals in the combined AG and KRG arm (n=26), median peripheral AI was 82 (77,88) % at baseline and 80 (72,89) % after 12 weeks. There were no significant differences from baseline to treatment-end within either group.

5.6.2.2 Central Augmentation Index

Central AI corrected for a heart rate of 75 beats per minutes was assessed at centre one (Table 5-6). Data analysis revealed that there was no significant effect of treatment on end values after adjusting for baseline values and BMI.
Table 5-6. The effect of combined AG and KRG (n=14) compared to placebo (n=12) on central augmentation index and indices of central blood pressure at centre one, n=26. Results are presented as unadjusted mean±SEM or median (IQR).

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO</th>
<th>AG+KRG</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central AI (%)</td>
<td>25 ± 2</td>
<td>24 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Central SBP (mm Hg)</td>
<td>110 ± 4</td>
<td>119 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Central DBP (mm Hg)</td>
<td>73 ± 3</td>
<td>78 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Central PP (mm Hg)</td>
<td>37 (29, 44)</td>
<td>37 (33, 47)</td>
<td>NS</td>
</tr>
<tr>
<td>Central MAP (mm Hg)</td>
<td>89 ± 3</td>
<td>96 ± 3</td>
<td>NS</td>
</tr>
</tbody>
</table>

5.6.3 Central Blood Pressure

Indices of the central pressure waveform were also evaluated at centre one (Table 5-6). This included central SBP, DBP, and mean arterial pressure (MAP). Statistical analysis revealed that there was no significant effect of combination ginseng use on adjusted means after 12 weeks of treatment administration. Although not significant, there was a trend toward lower central SBP, DBP and MAP by 3 to 5 mm Hg from baseline to treatment-end in the ginseng arm, while no change was observed in the placebo arm. Data for central pulse pressure (PP) was not normally distributed. Logarithmic transformation did not adjust the data to follow a normal distribution and therefore between-group medians were compared at week 12 using the Mann-Whitney U-test. No significant end-point differences were found. Medians for central PP were also compared from baseline to treatment-end within each group and also found not to be significantly different.
5.6.4 Ambulatory Blood Pressure

Ambulatory blood pressure was examined for 24 hours at baseline and treatment-end. End of treatment means measured in 19 subjects at centre one are presented in Table 5-7. Analysis revealed that there was no significant effect of treatment on end values adjusted for baseline values and BMI.

Table 5-7. The effect of combined AG and KRG (n=11) compared to placebo (n=8) on ambulatory blood pressure at centre one, n=19. Results are presented as unadjusted mean±SEM.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO</th>
<th>AG+KRG</th>
<th>P-VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean 24hr SBP (mm Hg)</td>
<td>118 ± 4</td>
<td>131 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Mean 24hr DBP (mm Hg)</td>
<td>74 ± 2</td>
<td>76 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Mean 24hr MAP (mm Hg)</td>
<td>89 ± 3</td>
<td>95 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean 24hr HR (mm Hg)</td>
<td>75 ± 3</td>
<td>76 ± 3</td>
<td>NS</td>
</tr>
</tbody>
</table>

SBP- systolic blood pressure; DBP- diastolic blood pressure; MAP- mean arterial pressure; HR- heart rate

5.7 Safety

5.7.1 Symptoms Report

The following is a list of side effects that were verbally reported by subjects during the treatment phase of the study. An increase in fasting blood glucose was reported after home monitoring assessment (n=3); two subjects were in the ginseng arm while one was in the placebo arm.

One subject in the placebo arm reported an increase in blood pressure assessed using their home monitoring device. One subject in each arm reported heaviness in the stomach while another subject in the ginseng arm reported constipation. A subject in the ginseng arm experienced
daytime nervousness, anxiety, hunger, shaky hands, hunger and tiredness. These symptoms would reportedly disappear in the evening. This subject was withdrawn due to undesired side effects and not included in analysis. Non-parametric analysis revealed that there were no significant differences in the frequency of reported side effects.

### 5.7.2 Biochemical Analyses

Statistical analysis comparing baseline to treatment-end revealed that there were no changes in the biochemical measures of safety shown in Table 5-8 and Table 5-9.

#### Table 5-8.

Comparing the effect of placebo to combined AG and KRG on hepatic and renal function from baseline to treatment-end, n=48. Results are presented as mean±SEM.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO (n=22)</th>
<th>AG+KRG (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WEEK 0</td>
<td>WEEK 12</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>24.1±1.6</td>
<td>22.6±1.5</td>
</tr>
<tr>
<td>Cr (µmol/L)</td>
<td>80.6±3.6</td>
<td>82.1±3.4</td>
</tr>
</tbody>
</table>

AST- aspartate aminotransferase; Cr- creatinine

#### Table 5-9.

Comparing the effect of placebo to combined AG and KRG on bleeding time from baseline to treatment-end at centre one, n=26. Results are presented as mean±SEM.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO (n=12)</th>
<th>AG+KRG (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WEEK 0</td>
<td>WEEK 12</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>30.3±0.3</td>
<td>30.1±0.3</td>
</tr>
<tr>
<td>PT (s)</td>
<td>11.0±0.2</td>
<td>10.8±0.2</td>
</tr>
<tr>
<td>INR</td>
<td>1.00±0.02</td>
<td>0.99±0.02</td>
</tr>
</tbody>
</table>

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

Reportedly, subjects did not change the dose, type of medications or forms of CAM that may affect their diabetes control throughout the study unless where indicated in section 5.3. Analysis of compliance revealed that there were no significant changes in diet and anthropometry from baseline to treatment-end.

5.8.1 Supplement Consumption

Raw data on supplement consumption was not obtained from the second centre at the time of analysis, however, it was indicated to be greater than >80% for all subjects. Capsule compliance (mean±SD) was 95±15% for the placebo arm, and 96±6% for the ginseng arm at centre one.

5.8.2 Anthropometry

As depicted in Table 5-10, there were no significant changes in anthropometric measures from baseline to treatment-end between treatment groups. This included weight, BMI, percent body fat and waist-to-hip ratios for both men and women.
Table 5-10. Comparing anthropometric changes between treatment groups from baseline to treatment-end, n=48. Results are presented as mean±SD.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO (n=22)</th>
<th>AG+KRG (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WEEK 0</td>
<td>WEEK 12</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>77.7±13.6</td>
<td>77.7±13.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.5±4.0</td>
<td>28.6±3.9</td>
</tr>
<tr>
<td>BF (%)</td>
<td>32±9</td>
<td>32±9</td>
</tr>
<tr>
<td>WHR (M)</td>
<td>0.96 ± 0.04</td>
<td>0.96 ± 0.06</td>
</tr>
<tr>
<td>WHR (F)</td>
<td>0.91 ± 0.09</td>
<td>0.91 ± 0.09</td>
</tr>
</tbody>
</table>

Wt- weight; BMI- body mass index; BF- body fat; WHR- waist-to-hip ratio; M- male; F- female

5.9.3 Diet Analysis

Analysis of three day diet averages depicted in Table 5-11 revealed that there were no significant changes in total caloric intake and macronutrient distribution from baseline to treatment-end in both treatment groups.

Table 5-11. Comparing diet composition between treatment groups from baseline to treatment-end at centre one, n=26. Results are presented as mean±SEM.

<table>
<thead>
<tr>
<th>OUTCOME MEASURE</th>
<th>PLACEBO (n=12)</th>
<th>AG+KRG (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WEEK 0</td>
<td>WEEK 12</td>
</tr>
<tr>
<td>Total Caloric Intake (kcal)</td>
<td>2295±310</td>
<td>1804±239</td>
</tr>
<tr>
<td>Carbohydrate Intake (g)</td>
<td>262±26</td>
<td>217±30</td>
</tr>
<tr>
<td>Protein Intake (g)</td>
<td>105±24</td>
<td>77±11</td>
</tr>
<tr>
<td>Fat Intake (g)</td>
<td>89±16</td>
<td>74±13</td>
</tr>
</tbody>
</table>
Chapter 6
Discussion and Conclusions

6.1 An Overview of the Results

The combined use of AG and KRG appears to be safe but did not demonstrate a statistically significant effect on metabolic and vascular parameters of efficacy when compared to placebo after 12 weeks of treatment. However, the clinical implications of the findings presented may still be relevant.

Considering all data, with the exception of the subject removed prior to analysis due to a change in diabetes medication, results indicated no significant end-difference in median HbA1c for those in the ginseng arm compared to those in the placebo arm. It is also important to note that all subjects in both groups continued their regular diabetes treatment, including diet, exercise and/or prescribed medication throughout the course of the study. Although not statistically significant, there was a 0.5% decrease from baseline in HbA1c for those in the ginseng arm with an end-difference in HbA1c of 0.7% between arms. There was a 0.2% increase from baseline in HbA1c observed for individuals in the placebo arm, which may, in part, be explained by the progressive decline in glycemic control in individuals with T2DM (131). Despite the lack of statistical significance, the results of the study presented here suggest that ginseng may show potential in maintaining, and possibly improving, glycemic control when compared to placebo.

Although insignificant, the 0.5% reduction in median glycated hemoglobin levels from baseline to treatment-end in the ginseng arm is five-fold the 0.1% reduction observed in the eight week AG crossover study conducted in 24 individuals with T2DM as part of the acute-to-chronic clinical testing program discussed in Chapter 2 (108). The improvement in HbA1c demonstrated in the crossover study was found to be statistically significant when compared to
the placebo arm (108). There was an insignificant increase in mean HbA1c for individuals in the placebo arm after eight weeks, a finding which is in line with the current investigation, which also demonstrated an observable, though not significant, HbA1c increase for individuals taking placebo (108). However, the end-difference between treatment arms in the current investigation, though not significant, was greater than twice that observed between the two arms of the AG long-term study, which found a 0.3% end-difference between the placebo treated group and the AG treated group (108). Conversely, a 12 week crossover study investigating the effects of 6g/day KRG in 19 individuals with T2DM failed to demonstrate a significant treatment effect in HbA1c from baseline to treatment-end and found that there was no difference in end values between the KRG arm and the placebo arm (10). Findings from this crossover study demonstrated an insignificant 0.1±0.4% (mean±SEM) decrease in HbA1c after 12 weeks in the placebo arm, and an insignificant increase in HbA1c by 0.1±0.4% observed for the KRG arm (10). Overall, these findings highlight the limited and inconsistent findings in the literature, yet also suggest that AG and KRG may demonstrate potential in improving glycemia. Further investigation is therefore required.

Furthermore, the results of this study demonstrated that the combined use of ginseng tends to improve office systolic blood pressure (BP) in a sample population with T2DM who, for the most part, already uses antihypertensive agents as indicated in Chapter 5. Although only approaching statistical significance (p=0.052), office systolic blood pressure decreased for individuals taking the combination ginseng treatment, with a demonstrated potential effect size (β [95% Confidence Interval]) of -8.3 [-16.6, 0.06] mm Hg as compared to placebo. When compared to the slight increase in BP observed for the placebo arm, and given an end-difference between treatment groups of 5±7 mm Hg (unadjusted mean±SEM), these findings are relevant in the context of blood pressure reductions observed for other therapeutic options. Although not
significant, the 7±6 mm Hg (mean±SEM) reduction in BP from baseline to treatment-end observed with combined AG and KRG administration demonstrated here is greater than that seen with lifestyle interventions, such as weight loss and physical activity; and may be considered comparable to reductions observed with antihypertensive medications (145-149). However, data analysis also revealed that the unadjusted end-difference for 24 hour ambulatory SBP was 13±6 mm Hg greater in the ginseng arm when compared to the placebo arm in a subset of these individuals (centre one). A closer examination of baseline values for ambulatory SBP revealed that baseline values were 9 mm Hg greater, though not statistically different, for individuals in the ginseng arm compared to those in the placebo arm. Accordingly, data analysis revealed that combined AG and KRG did not significantly affect end values for 24 hour ambulatory SBP when compared to placebo (p=0.8) and adjusted for baseline values and BMI.

Similarly, the unadjusted end-difference for central SBP was 9±6 mm Hg greater in the ginseng arm when compared to the placebo arm for individuals at centre one. However, higher baseline values in the ginseng arm once again contributed to this end difference and analysis revealed that there was no significant difference in end values when adjusted for baseline values and BMI (p=0.7).

The lack of a significant effect of ginseng on BP as demonstrated in the present study is comparable to previous studies in which ginseng administration was investigated for its effects on blood pressure. In a randomized, placebo-controlled, double-blind, crossover study conducted in 52 hypertensive individuals, 12 weeks of 3g/day AG administration did not significantly affect 24 hour ambulatory blood pressure (127). Likewise, 12 weeks of 6g/day KRG administration in 19 individuals with T2DM did not significantly affect office blood pressure or 24 hour ambulatory blood pressure when compared to placebo (10). Mean office SBP at study-end was
2±5 mm Hg (mean±SEM) mm Hg greater in the ginseng arm as compared to the placebo arm, but 2±5 mm Hg (mean±SEM) lower than baseline values (10). In addition, mean 24 hour ambulatory SBP was identical between both arms at study-end (10). A 2005 meta-analysis of RCTs revealed that a reduction in BP of 6/4.5 mm Hg decreases the incidence of major cardiovascular endpoints (146). The findings of the current study, although preliminary and only borderline significant, suggest that a comparable reduction in SBP may be attained through the use of combined AG and KRG. Therefore, the use of AG and KRG in combination may provide a novel therapeutic option in the management of hypertension and prevention of cardiovascular disease but requires further investigation.

A mechanistic explanation was not explored here; however, previous findings indicate that KRG, and specifically the ginsenoside Rg3, may improve vascular function through a nitric-oxide/cGMP mediated vasodilatory pathway (118,119). Compositional analysis of KRG revealed the Rg3 content in the current investigation to be 0.29% weight/weight (4.35mg/day) which is greater than the 0.05% (3mg/day) Rg3 content of the KRG extract used in our previous long-term study included in the acute-to-chronic KRG clinical testing program. Following KRG administration for 12 weeks in 19 individuals with T2DM, 3mg/day Rg3 did not significantly affect office blood pressure or 24 hour ambulatory blood pressure (10). This suggests that the greater Rg3 content of the KRG extract used in the current study may be responsible for the 5±8 mm larger reduction in SBP observed for individuals administered the ginseng treatment in the current study compared to that provided in previous studies.

It was originally postulated that, based on previously reported complementary actions of AG and KRG, their combination would result in glycemic improvements due to the potential for an additive or synergistic effect. The data presented here do not support this hypothesis; however,
there may be several underlying reasons for this which should be further explored. The potential for ginseng efficacy cannot be completely dismissed due in large part to the low statistical power inherent in the results. Furthermore, the subjects in this investigation had well-controlled glycemia and were taking the most efficacious medical therapies available. These were kept unchanged throughout the entire treatment period of the study. It is possible then that a significant improvement in HbA1c is lacking due to the well-controlled nature of the subject pool. On the other hand, it is possible that the combined use of AG and KRG may in fact have somewhat counteracting effects, or opposing actions, that ultimately result in an overall neutral effect. Alternatively, the composition of the investigated ginseng extracts may be ineffective due to their ginsenoside profile or other, unmeasured components.

6.2 Study Limitations

6.2.1 Sample Size

There are several limitations of this study which must be considered when interpreting the results. Statistical analysis of glycated hemoglobin, the primary outcome measure, failed to demonstrate a significant effect but the power associated with these results must also be considered. An unpaired T-test of end values between arms was used to provide an estimate of the power since this was not provided for the Mann-Whitney U-test. The power associated with the comparison of end values was only 0.22. Common statistical practice recommends a power of at least 0.80 in order to detect a reasonable departure from the null hypothesis. It seems that a difference between treatments may exist, however the sample size of the current study is inadequate. A larger sample may therefore be required to detect a significant treatment effect.
Nine subjects had not yet completed the study at the time of analysis and their data was therefore not considered here; inclusion of their data may affect the results. However, due to the large standard deviation in the results, which was determined to be 1.6% using T-test analysis, inclusion of data from an additional nine subjects may still be inadequate.

The observed improvement in brachial SBP is similarly limited in its interpretation. A standard deviation of 35 mm Hg is associated with the 5 mm Hg unadjusted end-difference between arms. This is considerably large, and primarily due to the differences in subjects between the two centres. A larger sample size may again provide clarity in the conclusions drawn from these results, which, at present, were considered borderline significant.

Furthermore, availability of equipment between centres played an important role in determining the total sample size. Centre one was the main centre and had a greater availability of resources, including access to the 24 hour ambulatory blood pressure monitoring devices and resources necessary to conduct the OGTT, while centre two did not. Centre two, therefore, did not conduct these tests. Once again, this limited the statistical power of the results.

### 6.2.2 Data Analysis

The baseline differences in subject characteristics between arms and centres were also a limitation in this investigation. Comparison of baseline characteristics between centres exposed a heavier (BMI, % body fat) subject pool at the second centre suggesting that these individuals would likely have a greater degree of insulin insensitivity. This sample pool also had significantly elevated blood pressure as compared to centre one and therefore a higher frequency of use of antihypertensive medication. These between-centre differences were also accountable
for some of the variation in the results. Nevertheless, an attempt was made to consider this variation in the statistical analysis of the results by adjusting for centre as a covariate.

### 6.3 The Yin and Yang Actions of Ginseng

The ginsenoside profile of AG and KRG can be considered inverse for certain ginsenosides and their ratios (69,70). Therefore, there exists a potential for AG and KRG to oppose one another in action, which may explain the neutral results observed in the present study. This potential for opposing actions can be demonstrated using compound K and Rg1 with their opposing effects on intestinal glucose absorption; where compound K is a PPD metabolite that has been demonstrated to enhance absorption, Rg1 reduces it (73). Given that AG generally has a greater PPD content and KRG has a greater PPT content (such as Rg1), the net effect of these ginsenosides may be neutral (50). This theory is consistent with the Yin-Yang concept in TCM where AG and KRG are believed to act as Yin and Yang –two opposing forces that together form part of the concept of holism (47). While each one on its own is effective, the combination may attain an equilibrium resulting in no effect, and this may offer an explanation for the findings presented here.

### 6.4 An Ineffective Ginseng Formulation

Conversely, AG and KRG may not oppose one other in action and the results in this investigation may be attributed to the specific formulation of the investigated product. The ginseng formulations used here are considered proprietary extracts of commercial products, thus our involvement and suggestions were limited. Although the basic method for extraction of the
most efficacious ginseng extract was suggested by us, further involvement was difficult due to confidentiality and availability of resources. Our suggested formulations and source of ginseng material were based on previous findings such that improvements in glycemia would be expected. However, the source of material and processing method chosen by the manufacturer may be responsible for the potentially ineffective formulations used here. Comparison of the current investigational material and that of previously determined efficacious preparations is therefore crucial in elucidating an effective component.

The acute-to-chronic clinical testing program conducted for both AG and KRG at the RFMC prior to this trial revealed indices of the ginsenoside profile that may be related to ginseng efficacy. The acute-to-chronic clinical testing program for AG found that its efficacy may be related to its total ginsenoside content and its PPD to PPT ratio, and the KRG program revealed that KRG efficacy may be related to its Rg1 content (9,109).

The effective formulation of AG was previously found to have 3.54% total ginsenosides and a PPD/PPT ratio of 2.4, while the total ginsenoside content of the AG in the current study was 5.04% and the PPD/PPT ratio was 4.14. Similarly, the Rg1 content of KRG previously found to improve PPG was 0.22% while it was only 0.17% in the current study. It is clear from Table 6-1 that the total ginsenoside content and PPD/PPT ratio for both AG and KRG used in the current investigation exceeds that of the previous efficacious preparations. Further examination reveals a comparative excess of PPD content, but a PPT content that is lacking. This may be indicative of an ineffective composition resulting from a critical ratio of PPD to PPT.
Table 6-1. The ginsenoside profile of AG and KRG used in the current investigation (combined AG and KRG) compared to profiles of previously determined efficacious ginseng preparations. Results are presented as mean of triplicate analysis in % weight per weight.

<table>
<thead>
<tr>
<th>Species</th>
<th>American Ginseng</th>
<th>Korean Red Ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
<td><strong>Current</strong></td>
<td><strong>Previous</strong></td>
</tr>
<tr>
<td>Ginsenoside</td>
<td>% (w/w)</td>
<td></td>
</tr>
<tr>
<td>Rg1</td>
<td>0.08</td>
<td>0.13</td>
</tr>
<tr>
<td>Re</td>
<td>0.90</td>
<td>0.91</td>
</tr>
<tr>
<td>Total PPT</td>
<td>0.98</td>
<td>1.04</td>
</tr>
<tr>
<td>Rb1</td>
<td>3.28</td>
<td>1.34</td>
</tr>
<tr>
<td>Rc</td>
<td>0.30</td>
<td>0.46</td>
</tr>
<tr>
<td>Rb2</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Rd</td>
<td>0.43</td>
<td>0.62</td>
</tr>
<tr>
<td>Total PPD</td>
<td>4.06</td>
<td>2.50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>5.04</td>
<td>3.54</td>
</tr>
<tr>
<td><strong>PPD:PPT</strong></td>
<td>4.14</td>
<td>2.40</td>
</tr>
</tbody>
</table>

Rg1, Re- conventional nomenclature for ginsenosides from the protopanaxatriol group of ginsenosides; Rb1, Rc, Rb2, Rd- conventional nomenclature for ginsenosides from the protopanaxadiol group of ginsenosides; PPT- protopanaxatriol; PPD- protopanaxadiols
Determining an effective therapeutic dose range is an important consideration for pharmacological therapies. Levels exceeding the prescribed efficacious range can be considered toxic or have a paradoxical effect, such as declining efficacy beyond a threshold, which is the case for metformin (14). A potential dose-response effect of ginseng administration was investigated in the acute-to-chronic clinical testing programs for AG and KRG and found to be non-existent. Although investigation of a ginsenoside dose-response effect would be included in such an investigation since the ginsenoside content would be fixed per gram of ginseng administered, the PPD:PPT ratio would remain unchanged. Varying the PPD:PPT content has yet to be investigated directly and may provide further insight into effective formulations of ginseng. Comparing a ginseng extract of PPDs to an extract of PPTs in their effects on glycemia may therefore be worth exploring.

Attributing the inefficacy of ginseng to its composition may be valid although discerning the individual components of the profile that are responsible is fairly difficult. This is partly because of the variability in the analytical methodology used to evaluate ginsenoside profiles. These differences were described in a 2004 study by Sievenpiper et al who found a coefficient of variation between 31-81% for assays used in ginsenoside quantification (68).

Ginsenoside analysis of material used in the current study was conducted by two separate laboratories, one of which used two different techniques. Each analysis was done in triplicate and showed large differences as depicted in Table 6-2. HPLC-UV was the technique employed at laboratory one and the first analysis performed at laboratory two. Laboratory two also performed an analysis using LC/MS/MS. The final profile presented in Chapter 4 was chosen for its comparability to previous studies using the same laboratory and analysis technique (i.e. analysis 2-1).
Table 6-2. The ginsenoside profile of AG and KRG as analyzed by two laboratories and two different analysis methods. Analysis is indicated as X-X, where 1-0= laboratory one, 2-0= laboratory two, 0-1= HPLC-UV, 0-2= LC/MS/MS. Results are presented as mean of triplicate analysis in % weight per weight.

<table>
<thead>
<tr>
<th>Species</th>
<th>American Ginseng</th>
<th>Korean Red Ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Source of Analysis</td>
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</tr>
<tr>
<td></td>
<td>Ginsenoside</td>
<td>% (w/w)</td>
</tr>
<tr>
<td></td>
<td>Rg1</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Re</td>
<td>2.04</td>
</tr>
<tr>
<td></td>
<td>Total PPT</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>Rb1</td>
<td>5.99</td>
</tr>
<tr>
<td></td>
<td>Rc</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td>Rb2</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Rd</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Rg3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total PPD</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>9.92</td>
</tr>
<tr>
<td></td>
<td>PPD:PPT</td>
<td>3.39</td>
</tr>
</tbody>
</table>

Rg1, Re- conventional nomenclature for ginsenosides from the protopanaxatriol group of ginsenosides; Rb1, Rc, Rb2, Rd, Rg3- conventional nomenclature for ginsenosides from the protopanaxadiol group of ginsenosides; PPT- protopanaxatriol; PPD- protopanaxadiols; 1-1 denotes laboratory one-HPLC-UV; 2-1 denotes laboratory two-HPLC-UV; 2-2 denotes laboratory two-LC/MS/MS
6.5 Caveats in Ginseng Research

This discussion forms the basis for larger caveats in ginseng research, particularly the compositional complexity of the plant and the plethora of existing sources of variability. In addition to analytical methods, these include variations in agricultural methods, manufacturing, and processing techniques. The inconsistencies in ginseng efficacy are often attributed to these variations and the findings of this investigation may support this claim.

6.5.1 Complex Composition

Elucidating the component of ginseng responsible for its efficacy has proven to be difficult. Although ginsenosides are considered the active components in ginseng, it is possible that the efficacy of ginseng may be related to other, unmeasured components. These components may act independently or interact with ginsenosides to affect glycemia.

Alternatively, the totality of its constituents may be responsible for ginseng’s beneficial properties. Potential interactions of ginseng constituents have not been evaluated to date; however, the totality of the root is investigated in studies administering unprocessed ginseng root, such as the acute studies in the acute-to-chronic clinical testing programs. This is unlike the current investigation which utilized ethanol extracts of ginseng.
6.5.2 Variability in Ginseng Composition and Analytical Methodology

Compositional variability of ginseng exists at a biological level, as well as a result of cultivation and processing methods, and subsequently, in methodologies used in its compositional determination.

The inter- and intraspecies variability of ginseng was previously discussed in Chapter 2. This can exist between different plant components and even in different root components of the plant (69,70,109). Variation also exists in the form of choice of ginseng species and ginsenosides evaluated in research; the coefficient of variation for species examined was found to be 26-103% and was 36-112% for indices of ginsenoside profile (68). These sources of variation reduce the comparability of ginseng research.

Agricultural, manufacturing and processing methods also contribute to variability in ginseng products. Evidence for this comes from an acute study in which five sources of AG were examined from farms in Ontario, Canada (107). Results found significant reductions in PPG following acute administration of ginseng material from three out of five farms but failed to demonstrate a significant effect for the others (107). Differences in nutrient availability, such as soil composition and moisture content, can affect ginseng composition and may be used to explain these results (51). Similarly, processing methods also contribute to variability and this can include differences in extraction method; from the technique employed to choice of solvent (70).

Regulatory authorities are attempting to reduce such sources of variation, and thus the potential for varied efficacy, by imposing good agricultural and manufacturing processes (150,151).
Health Canada has imposed regulations for manufacturing, while the Ontario Ginseng Growers Association (OGGA) has implemented strategies for good agricultural practice (150,151).

Furthermore, the Ginseng Evaluation Program (GEP), in which commercial ginseng products were profiled using one analysis technique, HPLC-UV, was launched by the American Botanical Council (ABC) in 1993. The results demonstrated substantial variation between ginsenoside profiles claimed on product labels to those uncovered in the program (51). The findings of the GEP lead to standardization of HPLC as the analytical method used in profiling labeled products of ginseng. However, differences in the assay used with this methodology still exist (68).

6.6 Future Directions

The growing popularity of natural products such as ginseng and the lack of evidence for their safety and efficacy warrant further research in this field. The results of this investigation emphasize the need to standardize and regulate ginseng preparations as well as to further examine the combined use of AG and KRG as discussed below.

6.6.1 Standardization of Ginseng

There is a need to enforce strict agricultural and manufacturing processes, and to standardize processing and analytical techniques in order to provide a basis for more consistent and comparable data and to minimize product variation. As mentioned, regulatory authorities such as the OGGA, Health Canada, and the ABC are currently trying to implement standard procedures in agricultural, manufacturing and analytical methods, respectively. Nevertheless, there is still a
need for strict regulations at the processing level and for international regulatory authorities to follow suit.

### 6.6.2 Future Studies

Although the combined use of AG and KRG investigated in the current study was not found to significantly affect parameters of metabolic control and blood pressure, the results of this investigation warrant further exploration of AG and KRG in combination. This could be done either through continued investigation of the current ginseng material, or, by selecting new material that may be more efficacious.

Investigation of the current AG and KRG extracts would require a larger sample size in order to detect a significant difference in HbA1c between treatment groups, if a difference does in fact exist.

The alternative is a step-wise approach analogous to the acute-to-chronic clinical testing program previously utilized in our clinic. This approach examined varied doses and preparations of ginseng in acute studies in order to elucidate the material most effective in improving glycemia. The most efficacious preparations, as determined by the acute studies, were then used to select the ginseng material that would be administered in a long-term study. Following this approach, the first step then, is to examine the effects of varying ethanol extracts of both AG and KRG individually, and compared to placebo. The most effective extract of each species could then be tested for combinatorial efficacy. The proportion of AG and KRG used in combination could be varied in order to try to determine the most optimal treatment. Varied proportions of AG and KRG would also test the proposition that AG and KRG may oppose each other in action,
resulting in an overall neutral effect. AG and KRG must therefore be assessed alone, in different combinations and compared to a placebo in order to elucidate their individual roles. Varied PPD/PPT ratios would be inherent in such a design owing to differences in the ginsenoside profiles of AG and KRG. This would help to address the observation that the PPD/PPT ratio of the ginseng extracts used in the current investigation are different from those of extracts previously found to have beneficial effects on glycemia. These studies would provide further insight into the roles of the PPD ginsenosides and the PPT ginsenosides in improving glycemia, as well as their relative contributions.

This series of testing could ultimately lead to a long-term, four-arm study testing AG and KRG alone, in an optimized combination of the two, and compared to a placebo, in order to evaluate the long-term metabolic and vascular effects of this combined treatment.

6.7 Conclusion

The hypothesis that the use of AG and KRG in combination would improve long-term glycemic control when compared to placebo was not supported by the findings presented here. However, the results did demonstrate an insignificant improvement in glycated hemoglobin. Similarly, the findings of the current study did not support the hypothesis that the combined use of AG and KRG would improve other glycemic parameters, blood lipids and blood pressure, but there was a trend toward lower office systolic blood pressure. Nevertheless, there exist several caveats and sources of variability in the presented research, and an additional nine subjects have yet to be considered in data analysis. The findings of this study did, however, support the hypothesis that the combined use of AG and KRG would not affect safety parameters. This finding is consistent
with previous literature and provides further safety evidence for ginseng use. In a society where
the use of CAM is flourishing, and given the potential for ginseng to provide a novel therapeutic
option in diabetes management and prevention of CVD, further investigation into ginseng and its
effects on metabolic control and blood pressure is therefore warranted.
References


Negatively Regulates Insulin Secretion and is a Major Link Between Obesity, Beta Cell Dysfunction, and Type 2 Diabetes. Cell 2001;105(6):745-755.


Appendix 1: Telephone Screening Questionnaire

GINSENG
In the research study of
Blood Glucose Control

Telephone Questionnaire

1. Where did you see the ad?
2. Are you between the ages of 40-75 years?
3. If female: are you post-menopausal? Y / N
   (post-menopausal includes those females with more than a year of cessation of
   menstruation).
4. Do you have diabetes? Y / N
5. How long ago were you diagnosed with diabetes?
6. Do you take insulin? Y / N (if Yes, cannot enroll in study)
7. Do you take any pills to control your diabetes? Y / N
   Specify: ____________________________ When did you start?
8. Do you take any other medications besides those for diabetes (eg. for cholesterol,
   triglycerides, etc.)? Y / N
   Specify: ____________________________ When did you start?
9. Besides your diabetes, would you consider yourself to be in general good health? Y / N
10. Do you smoke? Y / N
    If Yes, how much? ________________
11. Do you drink alcohol? Y / N
    If Yes, how much? ________________
12. Do you have any food allergies? Y / N
    Specify: ____________________________
13. Can you come to St. Michael’s hospital for clinic visits in the mornings (fasting) on a
    regular basis for the next 10 months? Y / N

We have an information session on Day: ___________ Time: ___________

Directions to St. Michael’s Hospital
70 Richmond street East, main floor, Risk Factor Modification Centre
Dr. Vuksan’s office (416) 864-5525

American and Korean
Ginseng: Long-term study
Subject #: _________
Appendix 2: Informed Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF RESEARCH STUDY

Long term Metabolic and Therapeutic effects of Combined treatment of American Ginseng (Panax quinquefolius L.) extract and Korean Red Ginseng (steamed Panax C.A. Meyer) extract in the treatment of Type 2 Diabetes (REB 07-116)

PRINCIPAL INVESTIGATOR
Alexandra Jenkins, PhD, RD
Post-Doctoral Fellow
Risk Factor Modification Centre
St. Michael's Hospital
Tel: (416) 867-7462
Email: alexandra.jenkins@utoronto.ca

CO-INVESTIGATORS

Vladimir Vuksan, PhD
Professor, Departments of Nutritional Science and Medicine
Faculty of Medicine, University of Toronto;
Associate Director, Risk Factor Modification Centre,
St. Michael's Hospital
Tel: (416)-867-7450
E-mail: v.vuksan@utoronto.ca

Dr. Robert Josse, MD, FRCPC (C)
Professor, Departments of Nutritional Science and Medicine
Faculty of Medicine, Univ of Toronto;
Tel: (416) 867-7455
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Dr. Lawrence Leiter, MD, FRCPC (C)
Head of Division, Professor
Department of Endocrinology and Metabolism
Faculty of Medicine, University of Toronto
St. Michael's Hospital
Tel: (416) 867-3696
Email: leiter@smh.toronto.on.ca

Ms. Jyoti Bhardwaj M.Sc. candidate
Department of Nutritional Sciences
Faculty of Medicine
University of Toronto
St. Michael's Hospital
Tel: (416) 864-6060 ext. 5527
Email: jyoti.bhardwaj@utoronto.ca

STUDY SPONSOR: Canadian Diabetes Association (CDA)

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 questions to a 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 the study doctor and/or personnel 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.
STATEMENT OF CONSENT

TITLE OF RESEARCH STUDY

Long term metabolic and therapeutic effects of combined treatment of American Ginseng (Panax quinquefolius L.) extract and Korean Red Ginseng (steamed Panax C.A. Meyer) extract in the treatment of Type 2 Diabetes

CONSENT

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

By agreeing to participate, I understand that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional duties.

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

Name of Participant                  Signature of Participant                  Date

I have explained the study to the above Participant the nature and purpose, the potential benefits, and possible risks associated with participation in this research study. I have answered all questions that have been raised.

Name of Person Obtaining Consent                  Signature of Person Obtaining Consent                  Date

I, Alexandra Jenkins, am the investigator responsible for the conduct of this study at St. Michael’s Hospital, and I have delegated the explanation of this study to

_____________________.

Signature of Principal Investigator                  Date
Appendix 3: Medical Information Form

**INFORMATION FORM**

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

<table>
<thead>
<tr>
<th>Gender:</th>
<th>Ht (cm):</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Male</td>
<td>Wt (kg):</td>
</tr>
<tr>
<td>□ Female</td>
<td>BF (%):</td>
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<table>
<thead>
<tr>
<th>DOB (dd/mm/yyyy):</th>
<th>Waist/Hip(cm):</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th>Age</th>
<th>SBP:DBP (mmHg):</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Yes □ No</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>High blood sugar</th>
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<tr>
<td>When: ________</td>
</tr>
<tr>
<td>How high:</td>
</tr>
<tr>
<td>Fasting glucose: _______ mmol/L</td>
</tr>
<tr>
<td>Post-meal glucose: _______ mmol/L</td>
</tr>
<tr>
<td>HbA1c (glycosolated haemoglobin): _______%</td>
</tr>
<tr>
<td>Rx: __________________________________________________________________</td>
</tr>
<tr>
<td>__________________________________________________________________</td>
</tr>
<tr>
<td>Complications: __________________________________________________________________</td>
</tr>
<tr>
<td>__________________________________________________________________</td>
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<table>
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<tr>
<th>High blood pressure</th>
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<tbody>
<tr>
<td>When: ________</td>
</tr>
<tr>
<td>How high:</td>
</tr>
<tr>
<td>sBP/DBP _______ / _______ mmHg</td>
</tr>
<tr>
<td>Rx: __________________________________________________________________</td>
</tr>
<tr>
<td>__________________________________________________________________</td>
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<tr>
<td>Complications: __________________________________________________________________</td>
</tr>
<tr>
<td>__________________________________________________________________</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>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.</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Yes □ No</td>
</tr>
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<table>
<thead>
<tr>
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</tr>
<tr>
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<td>□ Aunt/Uncle</td>
</tr>
<tr>
<td>Grandmother/grandfather</td>
<td>□ Grandmother/grandfather</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Do you take medications, herbs or supplements? If yes, then please describe, indicating types, brand names, doses, and times.</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ Yes □ No</td>
</tr>
</tbody>
</table>

| □ Grandmother/grandfather |

<table>
<thead>
<tr>
<th>Have you been diagnosed with any of the following? (If yes, please indicate onset date, treatment and therapy)</th>
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</thead>
</table>

version 6: June 6, 2008
American and Korean Ginseng / LT EXTRACT Study
<table>
<thead>
<tr>
<th>CONDITION</th>
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<tr>
<td>Stomach (gastric) ulcer</td>
<td></td>
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<tr>
<td>Duodenal ulcer</td>
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<tr>
<td>Intestinal parasites</td>
<td></td>
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<tr>
<td>Diarrhea (&gt; 2 liquid stools/day)</td>
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<tr>
<td>Constipation (≥ 3 days duration)</td>
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<tr>
<td>Heart disease</td>
<td></td>
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<tr>
<td>Stroke</td>
<td></td>
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<tr>
<td>Heart attack</td>
<td></td>
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<tr>
<td>Arrhythmia</td>
<td></td>
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<tr>
<td>Uncontrolled hypertension</td>
<td></td>
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<tr>
<td>Systolic BP ≥ 140</td>
<td></td>
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<tr>
<td>Diastolic BP ≥ 90</td>
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<tr>
<td>Blood clotting disorders</td>
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<td>Anaemia</td>
<td></td>
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<tr>
<td>Kidney disease</td>
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<tr>
<td>Psychiatric conditions</td>
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</table>

version 6: June 6, 2008
American and Korean Ginseng / LT EXTRACT Study
<table>
<thead>
<tr>
<th>CONDITION</th>
<th>NO</th>
<th>YES</th>
<th>Onset date</th>
<th>Present status</th>
<th>Active (please indicate treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious hepatitis (B, C, D)</td>
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<tr>
<td>Recently diagnosed infectious hepatitis A, E</td>
<td></td>
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<tr>
<td>HIV/AIDS</td>
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<tr>
<td>Tuberculosis</td>
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<tr>
<td>Cancer</td>
<td></td>
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<tr>
<td>Thyroid disease</td>
<td></td>
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<tr>
<td>Do you experience any of the following:</td>
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<tr>
<td>Fatigue</td>
<td></td>
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<tr>
<td>Unexplained weight gain</td>
<td></td>
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<tr>
<td>Dry skin and hair</td>
<td></td>
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<tr>
<td>Depressed mood</td>
<td></td>
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<tr>
<td>Cold intolerance</td>
<td></td>
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<tr>
<td>Constipation</td>
<td></td>
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<tr>
<td>Increased cholesterol?</td>
<td></td>
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<tr>
<td>Nervousness/irritability</td>
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<tr>
<td>Palpitations</td>
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<tr>
<td>Heat intolerance</td>
<td></td>
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<tr>
<td>Increased sweating</td>
<td></td>
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<tr>
<td>Unexplained weight loss</td>
<td></td>
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<tr>
<td>Insomnia</td>
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<tr>
<td>Pancreatic disease</td>
<td></td>
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<tr>
<td>Diabetes</td>
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<tr>
<td>Asthma</td>
<td></td>
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<tr>
<td>Any food allergies</td>
<td></td>
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<tr>
<td>Allergies to ginseng or cornstarch powder</td>
<td></td>
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<tr>
<td>Any food intolerance</td>
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</tbody>
</table>
Any other health problems?

□ No □ Yes (please describe):

________________________________________________________

________________________________________________________

________________________________________________________

Lifestyle and diet

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

________________________________________________________

Do you smoke? □ Yes □ No

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

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

________________________________________________________

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

________________________________________________________

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

□ < 3/day □ > 3/day

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

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

Type of coffee: ______________________

WOMEN ONLY:

Are you post-menopausal? □ Yes □ No

version 6: June 6, 2008

American and Korean Ginseng / LT EXTRACT Study
Did you recently experience any of the following symptoms?

<table>
<thead>
<tr>
<th>SYMPTOM</th>
<th>No</th>
<th>Yes</th>
<th>Onset date</th>
<th>Frequency</th>
<th>Duration</th>
<th>Severity (mild/moderate/severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloating</td>
<td></td>
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<tr>
<td>Belching</td>
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<tr>
<td>Flatulence</td>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Excessive urination</td>
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<tr>
<td>Nausea</td>
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<tr>
<td>Headache</td>
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<td>Dizziness</td>
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<tr>
<td>Insomnia</td>
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<tr>
<td>Anxiety</td>
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<tr>
<td>Disorientation</td>
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<tr>
<td>Poor wound healing</td>
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<tr>
<td>Excessive bleeding after cuts</td>
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<tr>
<td>Impaired vision</td>
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<tr>
<td>Heart flutters</td>
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<tr>
<td>Joint pain</td>
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<tr>
<td>Numbness</td>
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</tbody>
</table>
Have you participated in a clinical trial within the last 3 months? □ Yes □ No

Did you have blood sample drawn? □ Yes □ No

Did the nurses experience difficulty in drawing blood samples from you? □ Yes □ No:
□ finding veins □ problems of bleeding

Did you experience any discomfort during or after blood samples have been collected from you?
□ No

□ Yes (please describe): □ nausea □ fainting □ dizziness
other ________________________________
Appendix 4: Health Canada Notice of Authorization

May 30 2008 4:17PM NHPD

Health Canada

Health Products and Food Branch

Notice of Authorization

Company Code: 13740
File No.: 122380
Submission No.: 122380

May 29, 2008

Vladimir Vukcan, Ph.D.
Associate Director
Risk Factor Modification Centre
70 Richmond St. E.
Toronto, ON
M5C 1N8

Dear Dr. Vukcan:

Re: CLINICAL TRIAL APPLICATION for American and Korean Red Ginseng (extracts)
(Long Term Treatment) Natural Health Products Regulations Section: 67

The Natural Health Products Directorate, Bureau of Clinical Trials and Health Science, is pleased to inform you that the information and material to support the above Clinical Trial Application, have been assessed and we have no objection to your proposed study. Please consider this as your notice of authorization to sell or import a natural health product for the purposes of a clinical trial.

I would remind you of the necessity of complying with the Natural Health Products Regulations, Part 4, in the sale of this product for clinical testing. In addition, the Regulations (Part 4) impose responsibilities, including commencement notice, record keeping and reaction reporting, on those conducting clinical trials. Please ensure that all systems are compliant in order to meet these responsibilities.

You are also reminded that all clinical trials should be conducted in compliance with the Therapeutic Products Directorate’s Guideline for Good Clinical Practice.

Should you have any questions concerning this letter, please contact the submission coordinator, Claudia Bura at 613-941-6236.

Yours sincerely,

Robin J. Marles, Ph.D.
Director, Bureau of Clinical Trials and Health Science
Natural Health Products Directorate
2936 Baseline Rd., Ottawa, ON K1A 0K9

Canada
Appendix 5: St. Michael's Hospital Research Ethics Board Approval

Research Ethics Office
Telephone: (416) 864-6060 Ext. 2557
Facsimile: (416) 864-6043
E-mail:  rpadav@smoh.toronto.on.ca

June 06, 2008

Dr. Alexandra Jenkins,
Clinical Nutrition and Risk Factor Modification Centre
St Michael's Hospital

Dear Dr. Jenkins,

Re: REB# 07-116 - Long term Metabolic and Therapeutic effects of combined treatment of American Ginseng(Panax quinquefolius L) extract and Korean Red Ginseng (steamed Panax C.A. Meyer) extract in the treatment of Type 2 Diabetes

REB APPROVAL: Original Approval Date June 06, 2008
Annual/Interval Review Date June 06, 2009

Thank you for your application submitted on April 27, 2007. At the St Michael's Hospital (SMH) Research Ethics Board (REB) meeting held on , the above referenced study was discussed and subsequently the views derived from this discussion have been documented and resolved.

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

1. Protocol (ver. 4; 06 June 2008)
2. Advertisement (as submitted)
3. Consent Form (ver. 4; 06 June 2008)

Furthermore, the following documents have been received and are acknowledged:
1. Health Canada Notice of Authorization (NOA) [dated May 29, 2008]
2. Qualified Investigator Undertaking Form (dated April 2, 2007)
3. Data Collection Form (ver. 6; June 6, 2008)
4. Clinical Assessment Form
5. Symptoms Questionnaire

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.

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

Dr. Alexandra Jenkins (REB# 07-116)
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).

With best wishes

Dr. Julie Spence  
Chair, Research Ethics Board

Dr. Brenda McDowell  
Vice Chair, Research Ethics Board

JS/BJM/amli
ST MICHAEL’S HOSPITAL HEALTH SCIENCES RESEARCH PROGRAM
OFFICE OF RESEARCH ADMINISTRATION
Authorization Check List for Submission of Research Proposals and Grant Requests

Applicant(s): Dr. Alexandra Jenkins
Department: ____________________________

Funding Agency: ____________________________ Is this proposal: New __ Renewal __

Type of Grant: Operating __________, Equipment ________, Personnel ________, Other ____________

Full Title of Study: REB# 07-116 - Long term Metabolic and Therapeutic effects of combined treatment of American Ginseng (Panax quinquefolius L) extract and Korean Red Ginseng (steamed Panax C.A. Meyer) extract in the treatment of Type 2 Diabetes

(DUPLICATE STUDY TITLE - Need to change study title)

<table>
<thead>
<tr>
<th>Proposal:</th>
<th>Yes</th>
<th>No</th>
<th>If Yes, Reviewed by:</th>
<th>Pending Approval</th>
<th>Approved</th>
<th>Authorized By</th>
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<tbody>
<tr>
<td>Human Subjects to be used</td>
<td>✓</td>
<td></td>
<td>Research Ethics Board</td>
<td></td>
<td>✓</td>
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<tr>
<td>Biohazard Risk</td>
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<td>Safety Review Form</td>
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<td>Radiation Safety Officer</td>
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<tr>
<td>Animal Subjects to be used</td>
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<td>Inst Animal Care Committee</td>
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<tr>
<td>Does the Budget include:</td>
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<td>Research Vivarium Fee</td>
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<tr>
<td>Salaries/Benefits</td>
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<td>If Yes, Human Resource Review</td>
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<tr>
<td>Is space available to do this Research?</td>
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<td></td>
<td>If No, Space Allocation Committee Review</td>
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</tr>
<tr>
<td>Will the Proposed Research Involve the Following:</td>
<td>Yes</td>
<td>No</td>
<td>If Yes, Dept Head Review:</td>
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<tr>
<td>Nursing Services</td>
<td></td>
<td></td>
<td>Hematology Dept</td>
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<tr>
<td>Biochemistry Dept</td>
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<td>Anaesthesia Dept</td>
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<td>Pathology Dept</td>
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<td>Pharmacy Dept</td>
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<td>Med Art &amp; Photography</td>
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<td>Respiratory Services</td>
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<td>Other</td>
<td></td>
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<td>Other</td>
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<tr>
<td>Equipment Purchases:</td>
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<td></td>
<td>If Yes, attach Quotations</td>
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<tr>
<td>Equipment Maintenance:</td>
<td></td>
<td></td>
<td>If Yes, attach Quotations</td>
<td></td>
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</tr>
</tbody>
</table>

SMH RESEARCH PROGRAM ADMINISTRATION:

HOSPITAL OVERHEAD CHARGES: YES ______ NO ______

MANAGER, OFFICE OF RESEARCH ADMINISTRATION

DATE OF AUTHORIZATION: ________________

PLEASE SUBMIT ALL CONTRACTUAL AGREEMENTS FOR INSTITUTIONAL APPROVAL

FINANCE ACCOUNTS WILL NOT BE AUTHORIZED FOR RESEARCH PROPOSALS AND GRANT REQUESTS WITHOUT PRIOR COMPLETION OF THIS FORM.

THIS APPROVAL WILL BE VALID FOR A PERIOD OF 12 MONTHS FROM THE DATE OF AUTHORIZATION.
Appendix 6: University of Toronto Research Ethics Approval

PROTOCOL REFERENCE # 25449

July 26, 2010

Dr. Vladimir Vuksan
Nutritional Sciences and Medicine
University of Toronto
150 College Street
Toronto, ON M5S 3E2

Ms. Jyoti Bhardwaj
Nutritional Sciences and Medicine
University of Toronto
150 College Street
Toronto, ON M5S 3E2

Dear Dr. Vuksan and Ms. Bhardwaj:

Re: Administrative Approval of your research protocol entitled, "Long term Metabolic and Therapeutic Effects of Combined Treatment of American Ginseng (Panax quinquefolius L.) Extract and Korean Red Ginseng (steamed Panax C.A. Meyer) Extract in the Treatment of Type 2 Diabetes"

We are writing to advise you that the Office of Research Ethics has granted administrative approval to the above-named research study. The level of approval is based on the following role(s) of the University, as you have identified with your submission:

- Graduate Student research – hospital-based only
- Storage or analysis of De-identified Personal Information (data)

This approval does not substitute for ethics approval, which has been obtained from your hospital Research Ethics Board. Please note that you do not need to submit Annual Renewals, Study Completion Reports or Amendments to the ORE unless the involvement of the University changes so that ethics review is required. Please contact the ORE to determine whether a particular change to the University's involvement requires ethics review.

Best wishes for the successful completion of your project.

Yours sincerely,

S. Lanthier
Research Ethics Coordinator
Appendix 7: Clinical Assessment Form

CLINICAL ASSESSMENT

Subject #: __________

Date: __________________________

Treatment Code (circle one):

A

B

Anthropometry and BP

<table>
<thead>
<tr>
<th>Height (cm):</th>
<th>Weight (kg):</th>
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</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

Body Fat (%): _______

Waist: Hip (cm:cm): ______:_____

SBP/DBP (mmHg/mmHg): ______:_____

BG printout

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

Preclinical information

Did you consume at least 15g (1/2 oz) of carbohydrate 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, 1 mashed potato, 1 bowl of cereal with milk, 1 glass of juice, 1 drink, 1 orange or apple, 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

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

☐ Yes ☐ No

How many hours of sleep did you have last night? Does that represent a typical amount?

_______ hrs

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

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 ☐ Very Good ☐ Fair ☐ Poor
## Appendix 8: Symptoms Questionnaire

### SYMPTOMS QUESTIONNAIRE

Date: ________________________________

Date of last visit (dd/mm/yyyy): / /

**Treatment:**

<table>
<thead>
<tr>
<th>SYMPTOMS</th>
<th>PRESENCE</th>
<th>SEVERITY</th>
<th>DATES (mm/yyyy)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bloating</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Belching</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Flatulence</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Excessive urination</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Nausea</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Headache</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Dizziness</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Insomnia</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Disorientation</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Anxiety</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Poor wound healing</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Excessive bleeding after cut</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Impaired vision</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Heart flutters</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Joint pain</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Numbness</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
<tr>
<td>Other (specify):</td>
<td>□ Yes</td>
<td>Low</td>
<td>1-2-3-4-5-6-7-8</td>
<td>High</td>
</tr>
</tbody>
</table>
Appendix 9: Three Day Food Record

DAILY 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 weekdays.
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, TGBT, Campbell’s, Lipton, Becel
   - **Restaurant Names**
     Examples: McDonald’s, Wendy’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** (e.g. 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** (e.g. 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, each</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10cm x 10cm x 1cm</td>
</tr>
</tbody>
</table>

If you have any questions, please do not hesitate to contact us.
Clinical Nutrition and Risk Factor Modification Centre
70 Richmond Street East
Toronto, ON M5C 1N8
(416) 864-6060 ext 3366
### 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>
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<td></td>
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</tr>
</tbody>
</table>

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

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