ROLE OF NITRIC OXIDE IN THE TREATMENT OF TYPE 2 DIABETES WITH AMERICAN GINSENG (*Panax quinquefolius* L.)

by

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A thesis submitted in conformity with the requirements for the degree of M.Sc.
Graduate Department of Nutritional Sciences
University of Toronto

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ROLE OF NITRIC OXIDE IN THE TREATMENT OF TYPE 2 DIABETES WITH AMERICAN GINSENG (Panax quinquefolius L.)

Master of Science, 2000
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ABSTRACT

To investigate the role of nitric oxide (NO) in the treatment of diabetes with American ginseng extract (AG), a double-blind, placebo-controlled study was conducted in 24 type 2 diabetic subjects. They were randomized to take either AG 2.25g/day or placebo for 8 weeks, then crossover with at least 4 weeks washout. Blood pressure was determined and fasting blood was taken to assess NO, glycemic parameters and lipid peroxidation.

During AG treatment, NO increased significantly compared to placebo (P=0.031). The change was negatively correlated to HbA1c (R=-0.42, P=0.043), which decreased (P=0.026) following AG. Fasting blood glucose, lipid peroxidation and blood pressure were improved with AG (P<0.05 respectively).

It is concluded that in type 2 diabetes, AG reduced cardiovascular risk factors such as hypertension and improved glycemic control which was associated with NO enhancement.
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Most importantly, I would like to give thanks to God for his Grace and an abundance of blessings. Thank you for giving me the patience, vision, endurance, strength and helping me to maintain a sound mind throughout my academic career.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>x</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>2</td>
</tr>
<tr>
<td>2. LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>2.1. Ginseng</td>
<td></td>
</tr>
<tr>
<td>2.1.1. History</td>
<td>7</td>
</tr>
<tr>
<td>2.1.2. Chemistry</td>
<td>8</td>
</tr>
<tr>
<td>2.1.3. Pharmacokinetics and Pharmacology</td>
<td>10</td>
</tr>
<tr>
<td>2.1.4. Hypoglycemic action</td>
<td>11</td>
</tr>
<tr>
<td>2.1.4.1. Ginsenosides</td>
<td>15</td>
</tr>
<tr>
<td>2.1.4.2. Water (DPG-series) and methanolic (EPG-series) extracts of <em>F. ginseng</em></td>
<td>16</td>
</tr>
<tr>
<td>2.1.4.3. Ginseng polysaccharides: glycans called panaxans</td>
<td>17</td>
</tr>
<tr>
<td>2.1.4.4. Ginseng polypeptide</td>
<td>18</td>
</tr>
<tr>
<td>2.1.5. Cardiovascular actions</td>
<td>18</td>
</tr>
<tr>
<td>2.1.6. Side effect of ginseng.</td>
<td>19</td>
</tr>
<tr>
<td>2.2. Nitric Oxide</td>
<td></td>
</tr>
<tr>
<td>2.2.1. History</td>
<td>20</td>
</tr>
<tr>
<td>2.2.2. NO synthesis and physiology</td>
<td>21</td>
</tr>
<tr>
<td>2.2.3. Nitric oxide synthases (NOS)</td>
<td>23</td>
</tr>
<tr>
<td>2.2.4. Biotrasformation and analysis of NO</td>
<td>29</td>
</tr>
<tr>
<td>2.2.5. NO and pathophysiological conditions</td>
<td></td>
</tr>
<tr>
<td>2.2.5.1. Diabetes</td>
<td>30</td>
</tr>
<tr>
<td>2.2.5.2. Hypertension</td>
<td>31</td>
</tr>
<tr>
<td>2.2.5.3. Hypercholesterolemia and atherosclerosis</td>
<td>33</td>
</tr>
<tr>
<td>2.3. Ginseng and NO</td>
<td></td>
</tr>
<tr>
<td>2.3.1. Cardiovascular and antioxidant actions</td>
<td>35</td>
</tr>
<tr>
<td>2.3.2. Non-vascular actions</td>
<td>37</td>
</tr>
</tbody>
</table>
3. HYPOTHESIS AND OBJECTIVES

3.1. Hypothesis ................................................................. 40
3.2. Objectives ................................................................. 40

4. STUDY DESIGN AND METHODOLOGY

4.1. Subjects recruitment and profile ........................................ 42
  4.1.1. Inclusion criteria ..................................................... 42
  4.1.2. Exclusion criteria .................................................... 42
4.2. Preparation ............................................................... 43
  4.2.1. Powder form of AG .................................................. 43
  4.2.2. Placebo ............................................................... 44
4.3. Experiment design ........................................................ 44
4.4. Data collection and assays ............................................ 45
  4.4.1. Serum nitric oxide ................................................... 45
  4.4.2. Glycemic parameters ............................................... 46
  4.4.3. Lipid peroxidation .................................................. 46
  4.4.4. Blood pressure ...................................................... 46
  4.4.5. Anthropometry ..................................................... 47
  4.4.6. Food records ....................................................... 47
  4.4.7. Pill counts .......................................................... 47
4.5. Statistical analyses ..................................................... 47

5. RESULTS

5.1. Serum nitric oxide ...................................................... 50
5.2. Plasma HbA1c ........................................................... 53
5.3. Fasting blood glucose .................................................. 53
5.4. Serum insulin .......................................................... 56
5.5. Lipid peroxidation ...................................................... 56
5.6. Systolic blood pressure (SBP) ........................................ 59
5.7. Diastolic blood pressure (DBP) ....................................... 59
5.8. Blood pressure reclassification ....................................... 62
5.9. Body weight ............................................................. 64
5.10. Food record and pill count .......................................... 64
5.11. Correlation between serum NOx and other parameters ...... 67
5.12. Effect according to subjective symptoms ......................... 67

6. DISCUSSION AND CONCLUSIONS

6.1. Discussion ............................................................... 70
  6.1.1. Ginseng and Glycemic control: the role of NO ............... 70
    6.1.1.1. Modulation of digestion ..................................... 71
    6.1.1.2. Modulation of insulin sensitivity ......................... 71
    6.1.1.3. Modulation of insulin secretion ......................... 73
  6.1.2. Ginseng and blood pressure: the role of NO ............... 74
6.1.2.1. Direct modulation of NO generation .......... 76
6.1.2.2. Modulation NO generation through insulin action. 77
6.1.2.3. Modulation of NO level through less lipid peroxidation.... 79
6.1.3. Summary................................................................. 79
6.2. Conclusions............................................................... 80
6.3. Directions for future research................................. 80

7. REFERENCES CITED.......................................................... 82

8. APPENDIX................................................................. 96
LIST OF TABLES

Table 2.1. The clinical and pharmacological activities of ginseng .................................. 12

Table 2.2. Distinct NOS........................................................................................................ 27

Table 4.1. Nutrient profiles of AG.......................................................................................... 43

Table 5.1. Characteristics of 24 subjects with type 2 diabetes at baseline......................... 51

Table 5.2. Serum nitrates/nitrites (NOx) concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes.......................... 52

Table 5.3. Plasma glycosylated hemoglobin (HbA1c) concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes.................................................. 54

Table 5.4. Fasting blood glucose concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes............................................ 55

Table 5.5. Serum insulin concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes...................................................... 57

Table 5.6. Serum malondialdehyde concentration over 8 weeks treatment with American ginseng or placebo in 23 subjects with type 2 diabetes................................. 58

Table 5.7. Systolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes.................................................... 60

Table 5.8. Diastolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes.............................................. 61

Table 5.9. Number of subjects with type 2 diabetes in different blood pressure category at week 0 and week 8 with treatment of American ginseng or placebo........... 62

Table 5.10. Body weight over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes................................................................. 65

Table 5.11. Average daily intake of energy and macro nutrients over study period in placebo or American ginseng treatment................................................................. 66
LIST OF FIGURES

Figure 2.1. Structure of Ginsenosides........................................................................ 13

Figure 2.2. The pathway for the production of nitric oxide..................................... 26

Figure 2.3. Potential reactions of NO.................................................................... 26

Figure 2.4. Biotransformation of NO and its related N-oxides................................. 27

Figure 2.5. Current scheme for endothelium-dependent relaxation.......................... 28

Figure 4.1. Study schedule...................................................................................... 45

Figure 5.1. Serum nitrates/nitrites (NOx) concentration changes over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes............ 52

Figure 5.2. Changes in plasma HbA1c concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes................. 54

Figure 5.3. Fasting blood glucose concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes................. 55

Figure 5.4. Serum insulin concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes................................. 57

Figure 5.5. Changes of serum malondialdehyde concentration over 8 weeks treatment with American ginseng or placebo in 23 subjects with type 2 diabetes...... 58

Figure 5.6. Systolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes................................. 60

Figure 5.7. Diastolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes................................. 61

Figure 5.8. Number of subjects with type 2 diabetes in different blood pressure category at week 0 and week 8 with treatment of American ginseng................. 63

Figure 5.9. Number of subjects with type 2 diabetes in different blood pressure category at week 0 and week 8 with treatment of placebo. ......................... 63
Figure 5.10. Body weight over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. .................................................. 65

Figure 5.11. Correlation between the percent end difference of NOx and HbAlc....... 73
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>μmol/L</td>
<td>Micro-mole per liter (10⁻⁶ mol/L)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>Angiotensin-converting enzyme inhibitors</td>
</tr>
<tr>
<td>AG</td>
<td>American ginseng</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ARBs</td>
<td>Angiotensin receptor blockers</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>cNOS</td>
<td>Constitutive nitric oxide</td>
</tr>
<tr>
<td>DBP</td>
<td>Diastolic blood pressure</td>
</tr>
<tr>
<td>ecNOS</td>
<td>Endothelial constitutive nitric oxide synthase</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>g</td>
<td>Grams</td>
</tr>
<tr>
<td>GEP</td>
<td>Ginseng evaluation program</td>
</tr>
<tr>
<td>GLM</td>
<td>General linear model</td>
</tr>
<tr>
<td>HbA1c</td>
<td>Glycosylated hemoglobin</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>L-NAME</td>
<td>N⁰-nitro-L-arginine methyl ester</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>mg</td>
<td>Milligrams</td>
</tr>
<tr>
<td>mL</td>
<td>Milli-Litre</td>
</tr>
<tr>
<td>mmol/L</td>
<td>Milli-mole per liter ($10^{-3}\text{mol/L}$)</td>
</tr>
<tr>
<td>nNoS</td>
<td>Neuronal nitric oxide synthase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>NOx</td>
<td>Nitrate and nitrite</td>
</tr>
<tr>
<td>$P.\ ginseng$</td>
<td>$Panax\ ginseng\ C.A.\ Meyer$</td>
</tr>
<tr>
<td>$P.\ quinquefolius$</td>
<td>$Panax\ quinquefolius\ L.$</td>
</tr>
<tr>
<td>SBP</td>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
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</tbody>
</table>
CHAPTER ONE

INTRODUCTION
1. INTRODUCTION

From the previous National Health and Nutrition Examination Survey (NHANES II) in the US to the most recent (NHANES III), the prevalence of diabetes and intermediate classifications of hyperglycemia has increased (Harris et al, 1998). Combined these disorders now affect almost a third of the population aged 40-74 years: 14.3% for diabetes 15.6% for impaired glucose tolerance (IGT), and 9.7% for impaired fasting glucose (IFG), which overlaps with IGT (Harris et al, 1998). Canadian data paint a similar picture (Health Canada, 1999). The human and economic cost of this situation is considerable. Despite numerous preventive strategies and medications, diabetes and its complications remain prevalent. The need for more effective treatments is driving some people to herbal remedies. Over the last 7 years their consumption has increased by 380% in US (Eisenberg et al, 1998). One of the most popular is ginseng (Radimer et al, 2000). Growing evidence from in vitro, animal, and limited clinical data indicate that ginseng may influence carbohydrate metabolism and diabetes. American (Panax quinquefolius L) (Oshima et al. 1987), Chinese (Panax ginseng C.A. Meyer) (Ohnishi et al, 1996; Martinez and Staba, 1984), Korean (Panax ginseng C.A. Meyer) (Ohnishi et al, 1996; Martinez and Staba, 1984), and pseudo (Panax notoginseng) (Gong YH et al, 1991) ginsengs and their fractions (Oshima et al. 1987; Hikino et al, 1986) have been shown to possess significant hypoglycemic action in rodent models. Similar findings have also been noticed in the two human studies in this area. (Vuksan et al, 2000; Sotaniemi et al, 1995). The first found that chronic supplementation with a nonspecified type of ginseng resulted in an improvement in long-term glycemic control, although this
observation was complicated by significant weight reduction (Sotaniemi et al, 1995). In the second, our research group (Vuksan et al, 2000a) reported that American ginseng reduced postprandial blood glucose significantly when it was administered either before or together with a 25g oral glucose challenge compared to placebo in diabetic subjects and only when given before in normal subjects. Other unpublished observations from our clinic support these effects and have demonstrated a tendency toward higher insulin following the ginseng treatments compared to control. There nevertheless remains a deficiency of rigorously conducted controlled-clinical-trials on long-term American ginseng consuming to confirm either these hypoglycemic or other effects on risk factors in type 2 diabetes. Also the mechanisms of ginseng actions are unclear, although there is an extensive literature that deals with effects on carbohydrate and lipid metabolism and the cardiovascular system.

Ginseng is the common name of several species of the genus Panax of the family Araliceae indigenous both to Asia and North America. Major medicinally used ginseng species include Panax ginseng C.A. Meyer, found in North China, Korea, Manchuria, North Japan, and Siberia, commonly called Chinese or Korean ginseng; Panax quinquefolius, found in eastern North America, commonly called American ginseng (AG); Panax notoginseng Burk., found in south China, and commonly called San Chi or Tien Chi ginseng (Persons, 1994). All are similar in composition, but are believed to be somewhat different in their effect with some overlap. In-vitro and animal research findings are reported in more than 300 original papers in Chinese, English, and Russian (Liu and Xiao, 1992). Based on these studies Chinese and Korean ginsengs and some of their fractions have been noticed to affect blood flow and have anti-stress, memory
increasing, anti-fatigue, and anticarcinogenic activities. Other pharmacological properties include liver-protection, athletic endurance enhancement and immunostimulation in the rat. Its North American counterpart is thought to increase sex drive, memory, and learning; decrease aging; anticarcinogenic; possess digestion regulating; cardiovascular and liver protective activities in vitro and in the rat. Supporting clinical studies using ginseng of any type are few and inconclusive: some show an effect while others do not (Vogler et al. 1999).

Several recent studies have suggested that the antioxidant and organ-protective actions of ginseng are linked to enhanced nitric oxide (NO) synthesis in endothelium of lung, heart, and kidney and in the corpus cavernosum. Enhanced NO synthesis thus could contribute to ginseng-associated vasodilatation and perhaps also to an aphrodisiac action of the root (Gillis, 1997).

NO is a gas that transmits signals in the organism. Research results rapidly confirmed that NO is a signal molecule of key importance for the cardiovascular system and it was also found to exert a series of other functions. We know today that NO acts as a signal molecule in the nervous system, as a weapon against infections, as a regulator of blood pressure and as a gatekeeper of blood flow to different organs. NO is present in most living creatures and made by many different types of cells (Ellis G, 1998). When NO is produced by the innermost cell layer of the arteries, the endothelium, it rapidly spreads through the cell membranes to the underlying smooth muscle cells. Their contraction is turned off by NO, resulting in a dilatation of the arteries. In this way, NO controls the blood pressure and its distribution. It also prevents the formation of thrombi (Kelm, 1999). In carbohydrate metabolism, studies showed unclarified controversial
results. It is found in rat that early phase of glucose-stimulated insulin secretion is at least partly mediated by NO which could be produced in islet alpha and delta cells (Spinas et al, 1998), while others suggest NO is a potent inhibitor of glucose-stimulated insulin secretion (Mosen et al, 1999). More studies showed insulin stimulates the production of NO by human endothelial cells (Zeng and Quon, 1996; Baron 1996). Both hyperglycemia and dyslipidemia contribute to endothelial dysfunction. Hyperglycemia results in impairment of endothelial cell NO production (Sower and Lester, 1999). Type 2 diabetes is characterized by impaired endothelial dependent vasodilatation which may contribute to the high prevalence of vascular disease in such patients. Although hyperglycaemia, dyslipidaemia and hypertension can all independently cause a similar defect, recent data suggest that endothelial dysfunction may be intrinsic to the insulin resistance syndrome that commonly precedes type 2 diabetes. Such abnormalities in endothelial function could represent the impact of subclinical disturbance of metabolism or alternatively the presence of a common cellular defect that influences both nitric oxide bioavailability and insulin mediated glucose disposal (Tooke and Hannemann, 2000). The possibility that diabetes-induced endothelial dysfunction in diabetes results, in part, from a paradoxical increase in NO production during the course of the disease (Pieper, 1999). Since NO production is closely related with insulin resistance in patients with type 2 diabetes, we therefore investigated a possible influence of long term oral AG administration on NO level, glycemic control, lipid peroxidation, diabetic related cardiovascular risk factors such as hypertension, and their relationship in type 2 diabetes.
CHAPTER TWO

LITERATURE REVIEW
2. LITERATURE REVIEW

2.1. Ginseng

2.1.1. History

Ginseng is a generic term encompassing a wide variety of compounds derived for the root of the *Panax* species of the *Araliaceae* family. Specifically, ginseng is often applied to *Panax quinquefolius* L. (*P. quinquefolius*) and *Panax ginseng* C.A. Meyer (*P. ginseng*) (Li and Li, 1973). While *Panax* is derived from the Greek word, *pan-axos*, which literally means all-healing. Ginseng means 'essence of the earth in the form of a man' (Fulder, 1993).

North American ginseng is botanically known as *Panax quinquefolius* L., where L denotes Linnaeus who named the plant. Oriental ginseng (Chinese or Korean ginseng), on the other hand, was originally named *Panax schinseng* Nees by Nees van Esenbeck which was later changed to *Panax ginseng* C.A. Meyer by Carl Anton Meyer in 1842 (Fulder, 1991). Today, the Oriental variety is commonly referred to as *Panax ginseng* C.A. Meyer.

Canada is a natural habitat for American ginseng (AG). It is indigenous to the eastern areas of North America proliferating in the Ontario, Quebec, and Wisconsin. The Native Indians had used wild North American ginseng for its healing properties long before French colonization (Pritts, 1995). But in 1716, a Jesuit priest, Father Joseph-Francois Lafitau, documented the existence of AG in Canada. Exports of wild North American ginseng to China began in 1721 and their export value was second only to fur (Evans, 1994). After the introduction of AG to China, Chinese manuscripts and
traditional medical writing documented the benefits of AG consumption. The Chinese have since prized this herb for its healing and health promoting properties (Duke, 1989).

With time, the wild ginseng population became severely depleted from over-harvesting and commercial cultivation of AG in Canada began in the late 1890’s (Fisher, 1994). But it was not until the early 1980’s that ginseng production witnessed exponential growth due to the lure of lucrative profits. By 1998, the industry would see production reach more than 1800 metric tons (MT) compared to less than 25 MT in 1982. There are now more than 2000 hectares in production; about 1/3 in British Columbia and about 2/3 in Ontario. Export value has increased from just under $Cdn 3 million in 1982 to more than $Cdn 61 million in 1998 (Statistics Canada, 1999).

2.1.2. Chemistry

Different fractions of ginseng may play an important role in its effects. These include its polysaccharide, peptide, fatty acid, mineral, trace element, and ginsenoside profiles. Most pharmacological actions of ginseng, however, are attributed to the involvement of ginsenosides (Attele et al, 1999). These compounds belong to a family of steroids named steroidal saponins. The one exception is Ro. This ginseside is an oleanic acid-ginsenoside. The literature refers to them collectively as ginsenoside saponins, triterpenoid saponins, or dammarane derivatives. Some of the most common of these compounds and their classifications can be found in Figure 2.1.(Fuzzati et al, 1999).

Four main distinguishing features characterize the steroidal ginsenosides. The first feature is a four trans-ring rigid steroid skeleton that can be broken into two major classes, 20(S)-protopanaxadiols and 20(S)-protopanaxatriols (Attele et al, 1999). Their nomenclature is denoted from the attachment of two or three “R” groups respectively
(Figure 2.1.). Both are very similar to the classical steroid hormones. One exception is that panaxadiols and panaxatriols have a modified side chain at C-20 that is truncated in progesterone, cortisol, and aldosterone, or not present in estradiol and testosterone. Another exception is that these classes usually have a sugar residue attached to C-3, which in many steroids is an β-OH group. The second main feature of steroidal ginsenosides is their sugar moieties. Types of sugar moieties, their number, and their site of attachment impart considerable structural variation. Some sugar moieties present are glucose, maltose, fructose, and saccharose with sites of attachment including C-3, C-6, or C-20. Both the type of sugar and their site of attachment are thought to influence biological activity (Attele et al, 1999). The third major feature of steroidal saponins is their hydroxyl groups. The number and site of attachment of hydroxyl groups again impart considerable structural variation, with differences in the number of OH groups thought to influence pharmacological activity (Islam et al, 1997). The final important distinguishing feature of steroidal saponins is stereochemistry differences at C-20. The stereoisomeric possibilities at this site allow for functionally different chemical compounds that often differ in their potency, pharmacological activity, and pharmacokinetic profile. Most ginsenosides that have been isolated are naturally present as enantiomeric mixtures. Together these features define more than 20 types of ginsenosides: Ra1, Ra2, Ra3, Rb1, malonyl-Rb1, Rb2, malonyl-Rb2, Rc, Glc-Rc, malonyl-Rc, Rd, malonyl-Rd, Re, Rf, 20-Glc-Rf, Rf2, Rg1, Rg2, Rg3, Rh1, Rh2, Rh3, Rs4, and pseudoginsenoside F-11.

Methods for the purification and quantitation of ginsenosides by conventional High Performance Liquid Chromatography (HPLC), as well as electrospray HPLC and
mass spectrometry, are now available. Analysis revealed that the ginsenosides content depends on the species of ginseng, the manner of sample preparation, and the age and part of the plant extracted. Using conserved plant sequences as primers, the DNA sequences in the ribosomal ITS1-5.8S-ITS2 region have been amplified and determined for *P. quinquefolius* L. and *P. ginseng* C. A. Meyer. An authentication procedure based upon the restriction fragment length polymorphism in the region is able to differentiate between *P. quinquefolius* L. and *P. ginseng* C. A. Meyer (Ngan et al, 1999)

In 1993, American Botanical Council initiated a comprehensive study of commercial ginseng products sold throughout North America: the Ginseng Evaluation Program (GEP). GEP has analyzed more than 500 Asian, American, and Eleuthero (Siberian) ginseng products using HPLC to profile and assay ginsenosides and/or eleutherosides. From the GEP criteria, the chemical markers tested in both *P. ginseng* and *P. quinquefolius* are ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf and Rg1, Rg2. However, *P. quinquefolius* is identified by absence of Rf, very low Rb2 and no Rg2, and usually characterized by high Rb1:Rg1 ratio.

**2.1.3. Pharmacokinetics and Pharmacology**

Biotransformation of ginseng and ginsenosides are very complex. After intravenous injection of $^3$H-ginsenoside Rg1 in mice, a tri-phasic decline in blood radioactivity was observed. The blood radioactivity following oral administration of $^3$H-ginsenoside Rg1 peaked at 2.1 hours. The absolute bioavailability calculated from whole blood radioactivity following oral and intravenous administration was 49%. Study on the tissue distribution of $^3$H-ginsenoside Rg1 indicated that the radioactivity was decreased with the following order: kidney, adrenal gland, liver, lungs, spleen, pancreas, heart,
testes and brain. The protein binding was 24% in plasma, 48% in liver, 22% in testes and 8% in brain (Liu and Xiao, 1992).

Early studies revealed that ginseng possesses the biomodulatory effects on the higher centers of central nervous system, facilitating both physical and mental activities. It possesses a wide range of cardiovascular pharmacological activities including effects on heart, heart rate, blood pressure, vasulation and hemodynamics. It has a noteworthy effect on the endocrine system in regulating the blood sugar level as demonstrated in alloxan diabetics. Recent experimental and clinical studies concluded that it has a wide range of effects, such as remarkable anti-shock effect in circulatory failure, modulatory effects on the immune functions, modulation of cellular metabolic processes. modulation of neuroendocrine system activities, improvement of learning and memory processes, and aphrodisiac action (Table 2.1.) (Liu and Xiao, 1992).

2.1.4. Hypoglycemic action

In addition to their protective, immune, and aphrodisiac activities, there is a suggestion ginseng may influence carbohydrate metabolism and diabetes. AG (Oshima et al, 1987) had been shown to possess significant hypoglycemic action in rodent models. Very limited clinical evidence is available to confirm these findings. Only two published studies could be found that investigate the effect of ginseng on glucose regulation in humans. In the first, Sotaniemi and coworkers demonstrated improvements in fasting blood glucose and glycemic control as assessed by HbA1c in type 2 diabetic individuals treated with 100mg/day and 200mg/day of ginseng respectively relative to placebo following 8 weeks of treatment (Sotaniemi et al, 1995). The type of ginseng used in this
Table 2.1.

The clinical and pharmacological activities of ginseng

| Modulation of cardiovascular activities and anti-circulatory shock effects |
| Modulation of cellular metabolic processes on carbohydrate, fat and protein metabolism |
| Hypoglycemic effect |
| Antioxidative stress activity |
| Improvement of facilitation of learning and memory processes |
| Modulation of neuro-endocrine system activities |
| Promotion of hematopoiesis |
| Modulation of immune functions |
| Protection against radiation and liver toxicities |
Figure 2.1. Structure of Ginsenosides

<table>
<thead>
<tr>
<th>Ginsenoside</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh2</td>
<td>Glc</td>
<td>H</td>
</tr>
<tr>
<td>Rg3</td>
<td>Glc2-Glc</td>
<td>H</td>
</tr>
<tr>
<td>Rd</td>
<td>Glc2-Glc</td>
<td>Glc</td>
</tr>
<tr>
<td>Rc</td>
<td>Glc2-Glc</td>
<td>Glc6-Araf</td>
</tr>
<tr>
<td>Rb1</td>
<td>Glc2-Glc</td>
<td>Glc6-Glc</td>
</tr>
<tr>
<td>Rb2</td>
<td>Glc2-Glc</td>
<td>Glc6-Arap</td>
</tr>
<tr>
<td>Rb3</td>
<td>Glc2-Glc</td>
<td>Glc6-Xyl</td>
</tr>
<tr>
<td>Ra1</td>
<td>Glc2-Glc</td>
<td>Glc6-Arap4-Xyl</td>
</tr>
<tr>
<td>Ra2</td>
<td>Glc2-Glc</td>
<td>Glc6-Araf2-Xyl</td>
</tr>
<tr>
<td>Ra3</td>
<td>Glc2-Glc</td>
<td>Glc6-Glc3-Xyl</td>
</tr>
</tbody>
</table>

20S protopanaxadiol ginsenosides

Glc=β-D-Glucose
Rha=α-L-rhamnose
Arap=α-L-arabinose(pyranose)
Araf=α-L-arabinose(furanose)
Xyl=β-D-xylene
study however was not specified and the results were ambiguous due to significant weight loss differences between the treatment groups. In the only other clinical study in this area, Vuksan and coworkers investigated glycemic responses in ten normal and nine type 2 diabetic subjects after the administration of 3g AG or placebo given 40 minutes before (-40 minutes) or together with a 25g oral glucose challenge. It was observed that selected blood glucose concentrations and the area under the curve were reduced significantly when ginseng was administered either before or together with the challenge compared to placebo in the diabetic subjects and only when given before in the normal subjects (Vuksan et al, 2000a).

Data in press from our research group add further support to the hypoglycemic activity of ginseng. In three acute dose and time response studies (Vuksan et al, 2000b; Vuksan et al, 2000c; Vuksan et al, 2000d) in which we investigated a wide range of ginseng doses (1-9) and times of administration (0-120min before a glucose challenge), similar reductions in postprandial glycemia both in nondiabetic and diabetic subjects were observed. The first two studies showed that 3, 6, or 9g of AG compared to placebo reduced significantly the postprandial glycemic response to a 25g oral glucose challenge when administered 0 (together with), 40, 80, or 120 minutes before a glucose challenge in 10 diabetic subjects, but not when administered together with the challenge in 10 nondiabetic subjects. There were no differences observed in either study between the doses or the times of administration in their glycemic lowering effect. These data suggested that ginseng is equally effective at doses above 3g and when administered at any time together or before the challenge in diabetic subjects, but only when administered 40 minutes or more before the challenge in nondiabetic subjects. The third study showed
that 1, 2, or 3g of AG compared to placebo reduced significantly the postprandial glycemic response to a 25g oral glucose challenge when administered 40 minutes but not 20, 10, or 0 minutes before a glucose challenge in 12 nondiabetic subjects. Again there were no differences detected between the doses studied. The suggestion was that AG is equally effective at doses above 1g, but needs to be administered a minimum of 40 minutes before the challenge in nondiabetic subjects.

Although ginsenosides are considered to play a wide spectrum of pharmacological activities, it is interesting that some scientists stated that the various hypoglycemic and insulin-like principles of ginseng are other components in it.

2.1.4.1. Ginsenosides

In normal rats it was showed that a semi-purified saponin fraction from P. ginseng could stimulate various metabolic reactions involved in lipid and sugar metabolism (Yokozawa et al., 1985). More reports indicated that most of the biochemical actions of the semi-purified saponin extract might due to ginsenoside Rb2 (Yokozawa et al., 1984). Administration of Rb2 to streptozotocin-induced diabetic rats improved hyperglycemia, and the Rb2-treated diabetic rats had a significant rise of glucokinase activity in the liver and a significant decrease in glucose-6-phosphatase. This also associated with an increase in hepatic glycogen. As a result, it was concluded that Rb2 might elicit its hypoglycemic activity by changing the levels of gluconeogenic and glycolytic enzymes and shifting the direction of the overall metabolic flow toward glucose oxidation. Consequently, the work provided some evidence that ginsenoside Rb2 might be a useful hypoglycemic agent and that its action was very similar to the metabolic alterations produced by insulin.
2.1.4.2. Water (DPG-series) and methanolic (EPG-series) extracts of *P. ginseng*

In 80's Japanese scientists demonstrated that the water (DPG-series) and methanolic (EPG-series) extracts from *P. ginseng* had hypoglycemic activity in alloxan diabetic mice injected *i.p.* with the ginseng fraction. These extracts were first fractionated with a water-diethyl ether mixture, then the water layer was further treated with n-butanol, and the butanol layer was dialyzed. The outer dialyzates yielded the hypoglycemic extracts called DPG-3-2 and EPG-3-2. EPG-3-2 was reported to increase blood insulin levels in normal and diabetic mice (Kimura et al., 1981, Waki et al., 1982). The hypoglycemic activity of EPG-3-2 in diabetic mice was abolished by an insulin antiserum, suggesting a stimulatory action of the methanolic extract on blood insulin level. EPG-3-2 was found to modify the metabolic clearance of insulin. Simultaneous perfusion of the pancreas with DPG-3-2 and glucose was also shown to produce an additive effect on insulin release in both normal and diabetic rats. Cycloheximide did not diminish insulin release from perfused rat pancreas in the presence of DPG-3-2, implying that insulin release stimulated by the fraction was independent of insulin biosynthesis(Kimura et al., 1981). These findings seemed to suggest that some ginseng fractions could stimulate insulin release, especially glucose-induced insulin release from pancreatic islets, and thereby lowered blood glucose level (Ng and Yeung, 1985). Furthermore, although both EPG-series and DPG-series extracts had yet to be fully chemically characterized, chemical analyses on these extracts indicated that these ginseng fractions consisted of unknown substances as major components and ginsenosides as minor components, with EPG-series containing more saponins. However, neither one of
them was more effective in lowering blood glucose than the other, suggesting that the hypoglycemic effect of ginseng fraction might not be due to the saponins present as minor components (Kimura et al., 1981). Therefore, the hypoglycemic component might be a new principle different from ginsenosides.

2.1.4.3. Ginseng polysaccharides: glycans called panaxans

Ginseng polysaccharides were found to exhibit hypoglycemic activity. Konno et al. were the first to isolate these polysaccharides or glycans from an aqueous methanol/water extract of P. ginseng. There were currently ten hypoglycemic glycans recognized which were given the names panaxan A, B, C, D, E, Q, R, S, T, and U (Konno et al., 1984, 1985). Out of these 10 panaxans, only panaxan A was chemically characterized. $^1$H-NMR spectroscopy confirmed that panaxan A was composed mainly of $\alpha$-1→6 linked D-glucopyranose residues having branching at the C3 position. However, $^{13}$C-NMR spectrum suggested that these $\alpha$-glucose units were also linked at the 1, 3, and 6 position (Tomoda et al., 1984).

All of the panaxans were demonstrated to have hypoglycemic response when injected i.p. into normal mice. In addition, Panaxans A, B, and U, when administered i.p. in alloxan-induced hyperglycemic mice, lowered plasma glucose level (Oshima et al., 1985). The hypoglycemic capability of ginseng polysaccharides was further confirmed by Wang et al. as they found an association of ginseng polysaccharides with increases in adenosine-3',5'-cyclic monophosphate (cAMP) and adenylyl cyclase (AC). This action, however, was completely antagonized by propranolol, an adrenergic $\beta$-receptor inhibitor. In addition, both pyruvate and the activities of succinate dehydrogenase and cytochrome oxidase were found to increase, whereas the activity of lactate dehydrogenase decreased.
These findings suggested that the action of the polysaccharides extracted from *P. ginseng* was related to adrenergic receptors causing the production of cAMP which in turn activated the break down of glycogen for aerobic energy production. In fact, the authors believed that the polysaccharides might stimulate the release of insulin which led to a reduction in blood glucose and improvement of diabetes control (Wang et al., 1990).

2.1.4.4. Ginseng polypeptide

Ginseng polypeptide extracted from *P. ginseng* possessed similar effects as ginseng polysaccharides. When ginseng polypeptide was administered *i.v.* to rats, blood sugar levels and liver glycogen decreased. However, just like ginseng polysaccharides, this effect was inhibited by pretreatments of phentolamine and propranolol, suggesting that the effect of ginseng polypeptide on glucose metabolism might be related to adrenergic receptors. In addition, raised plasma glucose concentrations induced by adrenaline, glucose, or alloxan were ameliorated by ginseng polypeptide. At doses of ginseng polypeptide which caused decreases in blood glucose and liver glycogen, ginseng polypeptide also inhibited lactate dehydrogenase activity and stimulated the activities of the tricarboxylic acid cycle and respiratory chain enzymes such as succinate dehydrogenase and cytochrome oxidase, respectively. As a result, ginseng polysaccharides and polypeptides are very similar in their effects on hyperglycemia (Wang et al. 1990)

2.1.5. Cardiovascular Actions

Cardiovascular effects of ginseng root and individual ginsenosides have been studied extensively. Many reports describe transient vasodilator actions, in some cases followed by vasoconstriction and increase in blood pressure. The complex
cardiovascular actions of ginseng in vivo (intravenously to ten dogs) could reflect differing ginsenosides content of extract used or method of its extraction (Lee et al. 1981). Studies implicated the adrenergic nervous system in the cardiovascular effects of ginseng, and the panaxatriols, particularly Rg2, reduced Ach-evoked release of Catecholamines from bovine adrenal chromaffin cells. Extrapolating from these data, the authors suggest that ginseng may reduce elevated circulating catecholamine concentrations associated with various forms of stress in human (Tachikawa et al. 1995). Other studies indicated ginseng is used to treat coronary artery disease, myocardial infarction and reperfusion injury by its antioxidant actions. In different animal models, ginsenosides Ro and Rb protect against myocardial and cerebral ischemia/reperfusion damage. The protection is associated with an increase in 6-keto-PGF\(_{1\alpha}\), a stable metabolite of prostacyclin, and a decrease in malondialdehyde, a lipid peroxidation product (Chen X, 1996). According to the results in the different experiments, it is generally agreed that the protective effect of ginsenosides on myocardial ischemia is related to the following actions: (1) slowing heart rate, reducing oxygen consumption and decreasing peripheral blood vascular resistance; (2) improving myocardial metabolism, correcting sugar and fat metabolic processes; Other studies had been conducted to evaluate the inhibitory effects of ginseng on platelet aggregation, the rise of platelet cAMP level may be one of the mechanism (Liu and Xiao, 1992).

2.1.6. Side effects of ginseng

The LD50 of ginseng root in mice has been reported to be 10-30g/kg. Chronic treatment of rats, mice, dogs, and rabbits has shown very few observable signs of toxicity. In a 2-year study, 14 of 133 subjects reported nervousness, insomnia, and
gastrointestinal disturbance associated with prolonged oral consumption of doses that were much higher than those recommended. Also this study design in this case did not use a placebo (Siegel, 1979). As with clinical studies of efficacy, it is essential that side effects attributed to ginseng preparations must be accompanied by information on the content of ginsenosides. It is also notable that many reports of toxicity originate in those countries that lack medico-legal regulation of ginseng use and thus leave consumers exposed to variability in the quality of ginseng available. Reports of toxicity are rare in Germany and other European countries in which ginseng is medically prescribed and is used in recommended doses (Gillis, 1977).

2.2 Nitric oxide (NO)

2.2.1. History

Alfred Nobel invented dynamite, a product in which the explosion-prone nitroglycerin is curbed by being absorbed in kieselguhr, a porous soil rich in shells of diatoms. When Nobel was taken ill with heart disease, his doctor prescribed nitroglycerin. Nobel refused to take it, knowing that it caused headache and ruling out that it could eliminate chest pain. In a letter, Nobel wrote: “It is ironical that I am now ordered by my physician to eat nitroglycerin.” It has been known since last century that the explosive, nitroglycerin, has beneficial effects against chest pain. However, it would take 100 years until it was clarified that nitroglycerin acts by releasing NO.

NO is a gas that transmits signals in the organism. Signal transmission by a gas that is produced by one cell, penetrates through membranes and regulates the function of another cell represents an entirely new principle for signalling in biological systems. The
discoverers of NO as a signal molecule were awarded 1998 Nobel Prize. (Nobelförsamlingen Karolinska Institutet, 1998)

In 1977, Ferid Murad analyzed how nitroglycerin and related vasodilating compounds act and discovered that they release nitric oxide, which relaxes smooth muscle cells. He was fascinated by the concept that a gas could regulate important cellular functions and speculated that endogenous factors such as hormones might also act through NO. However, there was no experimental evidence to support this idea at the time. (Nobelförsamlingen Karolinska Institutet, 1998).

In 1980, Robert F Furchgott demonstrated in an ingenious experiment that acetylcholine dilated blood vessels only if the endothelium was intact. He concluded that blood vessels are dilated because the endothelial cells produce an unknown signal molecule that makes vascular smooth muscle cells relax. He called this signal molecule EDRF, the endothelium-derived relaxing factor, and his findings led to a quest to identify the factor. Louis J Ignarro participated in the quest for EDRF’s chemical nature. He performed a brilliant series of analyses and concluded in 1986, together with and independently of Robert Furchgott, that EDRF was identical to NO. The problem was solved and Furchgott’s endothelial factor identified. When Furchgott and Ignarro presented their conclusions at a conference in July, 1986, it elicited an avalanche of research activities in many different laboratories around the world. This was the first discovery that a gas can act as a signal molecule in the organism. (Nobelförsamlingen Karolinska Institutet, 1998)

2.2.2. NO synthesis and physiology
The formation of nitric oxide from L-arginine is now recognized as a ubiquitous biochemical pathway involved in the regulation of the cardiovascular, central, and peripheral nervous systems, as well as in other homeostatic mechanisms. The L-arginine: NO pathway comprises a substrate, L-arginine, a family of enzymes, the NO synthases (NOS), and at least one physiological effector system, the soluble guanylate cyclase. Nitric oxide is a colorless, odorless, hydrophobic, free radical gas with a half-life of 3-5 seconds that diffuses easily through cell membranes. Nitric oxide is synthesized from the amino acid precursor L-arginine by the enzyme nitric oxide synthase (Palmer et al, 1988) (Figure 2.2.). The reaction is a two-step process, yielding the intermediate product, N\textsuperscript{\textomega}-hydroxy-L-arginine and the final products NO and L-citrulline (Stuehr et al, 1991). Cofactors important to this reaction include flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), reduced nicotinamide adenine dinucleotide phosphate (NADPH), and tetrahydrobiopterin (THB) (Bredt et al, 1991). The electron acceptor of the transport reaction is dioxygen (Heinzel et al, 1992). The short half-life of NO is due to its rapid oxidation to nitrite (NO\textsubscript{2}\textsuperscript{-}) and nitrate (NO\textsubscript{3}\textsuperscript{-}) by reactions with O\textsubscript{2} and superoxide anion O\textsubscript{2}\textsuperscript{-}(Saran et al, 1990)(Figure 2.3.). In vivo reactions of NO can progress to the generation of peroxynitrite. This metabolite is formed with increased levels of O\textsubscript{2}\textsuperscript{-}, such as in inflammatory states. Peroxynitrite has a half-life of less than 1 second after which the protonated form ONOOH is formed (Beckman et al, 1990). ONOOH decomposes to NO\textsubscript{2} which then forms toxic radicals. Because of the many reactions NO may participate in, it becomes exceedingly difficult to measure accurately in vivo. One of the most common assays used to detect NO production is using NO analyzer to measure plasma or
urine nitrite plus nitrate, termed NOx, a stable final metabolites and an indirect determinant of NO production. (Ellis et al, 1998).

Within the vessel wall, the ultimate target of NO is soluble guanylate cyclase (sGC)(Waldman and Murad, 1988). Nitric oxide binds to the heme moiety of the enzyme, resulting in a conformational change and activation of sGC, causing an elevation of the second messenger cyclic guanosine monophosphate (cGMP) (Innarro et al, 1981). The result of this rise in cGMP is a relaxation of vascular smooth muscle cells and inhibition of platelet aggregation and adherence to the vessel wall. The mechanism by which NO causes vasorelaxation via cGMP is thought to be twofold: (1) a decrease in intracellular calcium and increase in the permeability of the potassium channels, leading to a hyperpolarization of the plasma membrane that prevents contraction and (2) activation of protein kinases leading to dephosphorylation of the myosin light chains, preventing the myosin/actin interaction (Moncada and Higgs, 1993). Other activators of the enzyme include the NO-containing vasodilator drugs used clinically which release NO and activate the enzyme in the same way.

2.2.3. Nitric oxide synthases (NOS)

Three distinct NOS isoforms of the enzyme responsible for the production of NO have been isolated and purified. (Table 2.2.)(Forstemann et al, 1993). These isoforms vary in subcellular location, amino acid sequences, regulation, and functional roles. The cDNA for each has been characterized, purified, and cloned. Each share an approximate 50%-60% amino acid sequence homology, especially at the carboxy-terminus end (Knowles and Moncada, 1994). All isoforms use L-arginine as the substrate to produce NO and L-citrulline. Two of the NOS isoforms are constitutive (always present) and are
termed cNOS. One cNOS has been localized to the endothelium (eNOS) while the other is found mainly in neurons (nNOS) as well as kidney macula densa, epithelium of the lung, and skeletal muscle. The third isoform of the enzyme is not expressed unless the cells have been stimulated (by certain cytokines, for instance) and is called inducible NOS (iNOS). Constitutive nitric oxide synthase produces NO at rest and is thought to be responsible for basal vascular tone (Luscher et al., 1993). Theoretically, most nucleated cells in the body have the capacity to express iNOS when stimulated, including vascular smooth muscle cells (Forstermann et al., 1993). The activation of eNOS and nNOS requires an influx of calcium ion into the cell and the binding of calmodulin to the enzyme and are therefore calcium-dependent (Cho et al., 1992). The iNOS isozyme is calcium-independent, since calmodulin remains bound to iNOS even in the presence of low amounts of intracellular calcium (Forstermann et al., 1994). The two nitric oxide synthases most closely associated with the vessel wall are eNOS and iNOS. The stimulation of the cell by acetylcholine, bradykinin, substance P, calcium ionophores, endothelial stretch, hypoxia and by platelet products such as thrombin, ATP and serotonin stimulate cNOS within vascular endothelium to release NO. The amount of NO produced is in the picomolar range and takes place within seconds (Forstermann et al., 1994). Endothelial NOS is felt to be responsible for the action of NO under physiologic conditions, such as, the maintenance of basal vascular tone. In contrast, iNOS is not expressed by the resting cell but requires stimulation by endotoxin and cytokines such as interferon-gamma, interleukin-1 (IL-1) and tumor necrosis factor (TNF) or shear forces upon the vascular wall (Chartrain, et al., 1994). Once induced, iNOS is active for a period of 4-24 hours during which it produces NO in nanomolar concentrations and therefore is
more important in pathophysiologic states (Forstermann et al., 1994). Agents such as glucocorticoids, transforming growth factor-β, interleukin-4, and interleukin-10 are known to inhibit iNOS (Morris and Billiar, 1994). The activity of iNOS seems to be consistent as long as the enzyme is detectable with the cofactor availability being the limiting step to the output of the system.
Figure 2.2. The pathway for the production of nitric oxide.

L-arginine $\overset{\text{NOS}}{\longrightarrow}$ NOS+THB $\overset{\text{NOS}}{\longrightarrow}$ N$\omega$-hydroxy-L-arginine $\overset{\text{NOS}}{\longrightarrow}$ L-citrulline +NO

NADPH  NADP  NADPH  NADP

NOS = nitric oxide synthase; NADPH = reduced nicotinamide adenine dinucleotide phosphate; NADP = oxidized nicotinamide adenine dinucleotide phosphate; THB = tetrahydrobiopterin.

Figure 2.3. Potential reactions of NO

$2\text{NO} + \text{O}_2 \leftrightarrow 2\text{NO}_2 \leftrightarrow \text{N}_2\text{O}_4$ (Blood)

$\text{NO}_2 + \text{NO} \leftrightarrow \text{N}_2\text{O}_3$ (Blood)

$\text{N}_2\text{O}_3 + \text{H}_2\text{O} \leftrightarrow 2\text{NO}_2^-(\text{Nitritite}) + 2\text{H}^+$ (Blood)

$4\text{NO}_2^- + 4\text{HbO}_2 + 4\text{H}^+ \leftrightarrow 4\text{NO}_3^- (\text{Nitrate}) + 4\text{Hb}^++ \text{O}_2 + 2\text{H}_2\text{O}$ (Erythrocyte)

$\cdot\text{NO} + \text{O}_2^- (\text{superoxide}) \leftrightarrow \cdot\text{OONO}$ (Peroxynitrite)

Metabolism of NO in human blood. NO released from endothelial cells into the vessel lumen is rapidly oxidized to nitrite in the plasma at the immediate site of its release. Nitrite is converted to nitrate via an autocatalytic reaction with oxyhemoglobin within the erythrocyte. Thus, nitrate represents the final, but rather unspecific end-product, whereas nitrite are specific, but rather unstable intermediates of NO metabolism in human blood.
Table 2.2. Distinct NOS

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Cells found</th>
<th>Time onset</th>
<th>Amount NO produced</th>
<th>Cofactors</th>
<th>Ca++ dependence</th>
</tr>
</thead>
<tbody>
<tr>
<td>ecNOS/eNOS</td>
<td>Endothelial cells</td>
<td>Seconds</td>
<td>+</td>
<td>NADPH THB, FAD FMN</td>
<td>Yes</td>
</tr>
<tr>
<td>nNOS</td>
<td>Neurons, skeletal muscle, macula dersa</td>
<td>Seconds</td>
<td>+</td>
<td>NADPH THB, FAD FMN</td>
<td>Yes</td>
</tr>
<tr>
<td>iNOS</td>
<td>Hepatocytes, neutrophils, chondrocytes, smooth muscle</td>
<td>4-6 hours</td>
<td>1000×</td>
<td>NADPH THB, FAD FMN</td>
<td>No</td>
</tr>
</tbody>
</table>

ecNOS/eNOS = endothelial nitric oxide synthase (constitutive); nNOS = neuronal nitric oxide synthase (constitutive); iNOS = inducible nitric oxide synthase; FMN = flavin mononucleotide; FAD = flavin adenine dinucleotide; NADPH = reduced nicotinamide adenine dinucleotide phosphate; THB = tetrahydrobiopterin.

Figure 2.4. Biotransformation of NO and its related N-oxides

Exogenously NOx (Intake/inhale)

Endogenously NO synthesized by NOS

Blood NO<->NOx

Saliva Nitrite->N2

Lung NO

Kidney NOx

Bowel NOx->NH3

Urine Nitrate or Urea cycle

Feces NOx/NH3

Air N2

NOx

NO
Figure 2.5. Current scheme for endothelium-dependent relaxation.

Agent A, acting on receptor (R) of endothelial cell activates Ca\(^{2+}\) influx, with the increase in intracellular Ca\(^{2+}\) activating through calmodulin the endothelial nitric oxide synthase (NOS), an oxygenase using L-arginine and NADPH as co-substrates, with FAD, FMN, and tetrahydrobiopterin as cofactors. NO diffuses to the smooth muscle cells where it activates guanylyl cyclase, with a resulting increase in cGMP, which initiates processes leading to relaxation. L-NMMA and L-NAME are arginine derivatives which inhibit NOS, and O\(^{2-}\) and HbO\(_2\) are potent scavengers of NO. PDE5: Phosphodiesterase type 5.
2.2.4. Biotransformation and analysis of NO

The steady-state concentration of NO is determined by its rate of formation and its rate of decomposition. A proper knowledge of the metabolism of NO is requisite for correct understanding and evaluation of its biological effects, and also for the development of reliable analytical methods of assess the production of NO in biological systems. Kelm et al reviewed the metabolic pathway of NO in the intact organism depends strongly on its site of administration and on its site of formation. Experimental and clinical data on the most relevant metabolic pathways of endogenously formed NO and on the biotransformation of exogenously applied NO and its related N-oxides (Figure 2.4.) (Kelm, 1999).

There are many methods of measuring nitrite and nitrate in plasma such as colorimetric methods; ultraviolet spectrophotometric method; methods using electrodes or bioanalytical elements; methods using NO analyzers; capillary electrophoresis; gas chromatography and gas chromatography-mass spectrometry; and high-performance liquid chromatography (HPLC) (Ellis et al, 1998). Nitrite is difficult to measure reliably in blood because it is unstable, being rapidly oxidized to nitrate. When added to blood, 16% of added nitrite remained after 30 min at room temperature; at 60 min, the corresponding fraction was <5%. Once plasma is separated from blood (avoiding hemolysis), both nitrite and nitrate are stable at −20°C for at least 1 year (Moshage et al, 1995). Nitrite concentrations in plasma are usually only a small fraction (<5%) of the nitrate concentrations, reflecting in part the dietary intakes of these ions and also their bioreactivity. A combined measurement of nitrate plus nitrite (NOx) is often used with
NO analyzers because the procedures can be simplified and the stringent sample preparation requirements for nitrite analysis can be avoided.

NO analyzers have been designed to measure NO in air samples by chemiluminescence in the presence of ozone. In order to measure nitrite or nitrate, the NO analyzers are coupled to an enclosed vessel through which an inert gas, such as nitrogen or helium, is purged (Cox, 1982). Then selective chemical reduction of either nitrite or nitrate to nitric oxide can be monitored directly as sequential samples are added to excess of the appropriate reagent. Acetic acid/iodide are often used for nitrite or vanadium II/NaOH for nitrate. Various oxidants have been tested and compared (Yang et al, 1997). It is subject to interference by a number of arginine analogs currently investigated as potential pharmacological agents. The method has also been applied to saliva and urine.

There is no publications which states what a standard level for NOx in Plasma might be. NOx levels in human plasma can change depending on dietary intake of NO producing foods. Tsikas et al reviewed 12 studies that measured plasma and urine samples from healthy humans by different assay method reported in the literature, the nitrate concentration varied from 15 to 68 μM and nitrite from 0.14 to 4.2 μM (Tsikas et al, 1997)

2.2.5. NO and pathophysiologic conditions

From diabetes to hypertension, cancer to drug addiction, stroke to intestinal motility, memory and learning disorders to septic shock, sunburn to anorexia, male impotence to tuberculosis, there is probably no pathological condition where nitric oxide does not play an important role.
2.2.5.1. Diabetes

In diabetes mellitus there is a generalized vasculopathy which is associated with an impaired NO-dependent vasorelaxation. The endothelium-dependent relaxations between Type 1 and 2 diabetics are different. In the Type 1 diabetic, the vasodilatory defect seems to be related to the vascular smooth muscle being less responsive to endothelial-generated NO and not a dysfunction in the endothelial production of NO. (Calver et al, 1992). In the Type 2 diabetic the predominant evidence suggests both endothelial and smooth muscle cell dysfunction. The reason for these differences is not completely understood. The mechanism of impaired function seems to be related to the hyperglycemic state. When an animal is manipulated chemically to induce diabetes there is an impaired endothelium-dependent relaxation with a diminished level of cGMP (Kamata et al. 1989). Mechanistically, when serum glucose levels are elevated, glucose metabolism is shunted to the conversion of sorbitol which in turn is converted to fructose. The conversion of glucose to sorbitol is via the aldose reductase enzyme. Aldose reductase is present in large amounts in the endothelial cell. It has been suggested that the increased levels of sorbitol act osmotically to cause the endothelial cell to swell and act adversely upon endothelial-dependent relaxation. Interestingly, the impairment of endothelial function in diabetes has been reversed when aldose reductase inhibitors are administered(Greene et al, 1987). Other derangements of the endothelium with diabetes include an enhanced generation of endothelium-derived contracting factor (Tesfamariam, 1990). Further, NO is known to degrade in the presence of superoxide anions. The overload of glucose metabolism in diabetes generates an abundance of oxygen-free radicals all of which may influence NO prior to the diffusing to the smooth muscle cell.
Ham and co-workers had demonstrated impaired endothelium-dependent relaxation in diabetic, renal disease patients prior to combined kidney/pancreas transplantation with return of normal function after correction of the diabetic state (Ham et al, 1996). This study, among others, demonstrates that the effects diabetes has upon the vascular wall is reversible.

Functional data from Sweden (Panagiotidis et al. 1992a, 1994, 1995, Akesson & Lundquist 1996, Salehi et al. 1996, Akesson & Lundquist 1998, Henningsson et al. 1999) have unambiguously suggested that NO negatively modulates insulin secretory processes induced by L-arginine, glucose, sulphonylurea and cholinergic stimulation, whereas NO, in contrast, is stimulatory to glucagon release. Similar results regarding insulin secretion were reported from other laboratories (Cunningham et al. 1994, Gross et al. 1995, Antoine et al. 1996, Sjoholm 1996), whereas no effects (Jones et al. 1992) or even a stimulatory action by NO on insulin release (Laychock et al. 1991, Schmidt et al. 1992, Laffranchi et al. 1995, Willmott et al. 1995, Spinas et al. 1998) have been observed. Hence, there is presently no consensus as to whether NO in fact inhibits, stimulates, or has no appreciable effect on the insulin secretory mechanisms. This might be due to different experimental conditions such as incubation of islets in high or low glucose, the use of different NOS inhibitors or different types of extracellular and intracellular NO donors, as well as the use of intact islets versus β-cell lines.

However, insulin has been demonstrated to stimulate production of NO in endothelial cells (Zeng and Quon, 1996). It has several direct vascular actions that contribute to either vascular protection or injury, depending on the cell type. Vascular protective effects of insulin include stimulation of endothelial cell production of the
vasodilator NO. This, in turn, inhibits formation of lesions dependent on migration and proliferation of vascular smooth muscle cells, attenuates binding of inflammatory cells to the vascular wall, and inhibits thrombosis by reducing platelet adhesion and aggregation. One of the major pathways of insulin action is the phosphatidylinositol 3-kinase pathway, which is important for glucose transport in skeletal muscle, as well as endothelial NO production and insulin-induced vasodilation (IIseh and Law, 1999)

2.2.5.2. Hypertension

Hypertension is a significant risk factor in the development of vascular disease. NO production is diminished within the vessels and platelets of patients with hypertension. Endothelium-dependent vasorelaxation is impaired in hypertensive animal models and humans. The exact mechanism remains unclear. Substantial evidence demonstrated that NO plays critical role in the maintenance of blood pressure homeostasis (Rees at al, 1989). Treatment with eNOS inhibitors (Moncada and Higgs, 1991) and disruption of the gene encoding eNOS (Huang et al, 1995), significantly increased blood pressure in rats and mice, respectively. Shear stress as well as locally generated compounds such as bradykinin, acetylcholine, ATP were reported to be stimulators for endothelial NO production (Milner et al, 1990). There has been clear evidence of an association between hypertension and endothelial dependent relaxation of coronary arteries (Egashira et al, 1993). In vivo experiments have demonstrated the endothelium-dependent vasorelaxations are impaired in patients with essential hypertension (Panza et al, 1990). Interestingly, these experiments concluded that the impaired vasorelaxation is caused by an endothelial mechanism in that when NO-producing drug nitroprusside was infused the vasorelaxation was no different than
normotensive patients. This means that the soluble guanlylate cyclase and cGMP pathway in the smooth muscle cell is likely not to be affected but rather there is a deficit in NO production. John and Schmieder concluded that the bioavailability of nitric oxide is probably impaired not by a single defect, but by various mechanisms affecting nitric oxide synthesis as well as nitric oxide breakdown. In hypertension, increased superoxide anion production and oxidative stress represents a major mechanism (John and Schmieder, 2000). Decreased bioavailability of nitric oxide does not only impair endothelium-dependent vasodilation, but also activates other mechanisms that play an important role in the pathogenesis of atherosclerosis.

2.2.5.3. Hypercholesterolemia and atherosclerosis

Hypercholesterolemia was the first pathological condition shown to be associated with an impaired endothelium-dependent vasorelaxation both in animals and man (Jayakody et al, 1985; Ludmer et al, 1986). Hypercholesterolemia impairs endothelium-dependent vasodilation not only in large conductance vessels. Several investigations demonstrated a similar effect in the microcirculation (Zeiher et al, 1991). A major mechanism underlying impaired endothelium-dependent vasodilation in hypercholesterolemia is thought to be the destruction of readily formed endothelium-derived NO by excess ambient levels of superoxide (Kojda and Harrison, 1999). It was found that eNOS dependent superoxide production was enhanced on human endothelial cells incubated with native low density lipoprotein (LDL) (Pritchard et al, 1995). Therefore it is assumed that native LDL uncouples L-arginine metabolism from NO release to increase eNOS-mediated generation of superoxide. It is well known that many cellular and subcellular events such as cellular growth and vasomotor function are abnormal in atherosclerotic
lesions. There is abundant evidence reported that the endothelial NO pathway is also involved in atherosclerosis. In patients with coronary atherosclerosis basal NO release was impaired, while L-arginine improved endothelium-dependent relaxation and restored endothelial dysfunction in the coronary microcirculation (Cooke et al, 1992). It is well known an early event in the formation of an atherosclerotic plaque is the deposition of platelets. NO has been shown to inhibit mitogen release from platelets and thereby reduce proliferation of the intimal layers. With the development of atherosclerosis, NO activity is impaired in a number of vascular beds in animals and in human atherosclerotic coronary arteries (Jayakody et al, 1987, Forstermann et al, 1988). This impairment of endothelial-dependent vasodilation is reversed in hypercholesterolemic by the exogenous administration of the NO precursor L-arginine accompanied by a marked inhibition of vascular plaque formation. Additional support for the protective role of NO in atherosclerosis is provided by animal studies demonstrating accelerated plaque formation with chronic inhibition of the NO synthase enzyme by Nω-nitro-L-arginine methyl ester (L-NAME). The exact mechanism for these findings has yet to be fully elucidated (Dattilo and Makhoul, 1997).

2.3. Ginseng and NO

2.3.1. Cardiovascular and antioxidant actions

Much evidence points to a close link between damaging actions of free radicals of oxygen and many forms of human disease, including cardiopulmonary pathology and reperfusion ischemia in heart and lung. In the circulatory system, the vascular endothelial cell is an early focus of free radical injury. Kim and co-workers found
ginsenoside reduced the pulmonary edema that follows free radical injury, and this effect was eliminated by nitro-L-arginine, and inhibitor of NOS. The conversion of L-arginine to L-citrulline in confluent bovine aortic endothelial cells in culture was enhanced significantly by ginseng. It was proposed that ginseng causes vasorelaxation and prevents manifestations of oxygen free radical injury by promoting release of NO (Kim et al, 1992).

The cardioprotective action of ginseng against ischemia-reperfusion injury has been reported in a study of patients undergoing cardiopulmonary bypass for mitral valve surgery. Total ginseng extract enhanced recovery of cardiac hemodynamic performance and significantly lowered mitochondria swelling during the period of ischemia (Zhan et al, 1994). In vitro Kim et al. found that vascular relaxations induced by ginsenosides are mediated by release of endothelium-derived nitric oxide which enhanced the accumulation of cGMP in rat aorta (Kim, 1994), while Nakajima et al found ginseng promoted the proliferation of cultured human vascular endothelial cells and promote physiological activities (Nakajima, 1998).

Endothelial damage is considered to be the initial step in the genesis of thrombosis and arteriosclerosis. Yuan and co-workers evaluated the protective effects of American ginseng extracts on endothelial cell injury and thrombin-induced endothelin release. The results suggest pharmacological action of P. quinquefolius is partially due to NO release, and may play a therapeutic role in facilitating the hemodynamic balance of vascular endothelial cells (Yuan et al, 1999).
2.3.2. Non-vascular actions

Support for the proposal that ginseng might enhance NO synthesis comes also from experiments with non-vascular system. A single i.p. injection of ginseng extract increased nitrites, nitrates, and cGMP level in rat serum and urine (Han and Kim, 1996). These effects were reversed by inhibition of NO synthase and restored by L-arginine. Similar action was seen with rat kidney, isolated glomeruli, and cortical tubules and was blocked by inhibition of NOS. The authors concluded that ginseng extract stimulates NO production in the kidney and thus may protect against ischemia by increasing renal blood flow (Han and Kim, 1996). Kang et al. found that addition of ginseng to a high cholesterol diet fed to rabbits preserved most of the normal dilatation of preconstricted aortic rings in response to acetylcholine (Kang et al. 1995).

Ginseng has long been reported to have aphrodisiac properties. Recognition of the importance of NO in the mechanism of penile erection led to consideration of a possible ginseng-NO link using the corpus cavernosum of the rabbit as a model. Chen and Lee found that ginseng extract relaxed the corpus cavernosum in a concentration-dependent manner. The authors concluded that ginsenosides may release NO from endothelial cells and perivascular nitrergic nerves in the corpus cavernosum and speculated that the aphrodisiac effect of ginseng may be linked, in part, to release of NO (Chen and Lee, 1995).

2.3.3. Summary

Recent studies suggest that some effects of ginseng in experimental system may be explained by the enhanced presence of NO. Evidence has been offered that (1) ginseng enhances formation of citrulline from added arginine, implying enhances
systhesis of NO, (2) known inhibitors of NO synthase such as L-NAME, block both the action of ginseng on citrulline formation from arginine and Ach-induced vascular relaxation of preconstricted tissue, (3) when used, arginine reverses the action of NO synthase inhibitors, and (4) when measured, tissue cGMP has been increased by ginseng (Gillis, 1997).
CHAPTER THREE

HYPOTHESES AND OBJECTIVES
3. HYPOTHESES AND OBJECTIVES

3.1. Hypotheses

In type 2 diabetes:

1. Glycemic control will be improved after long-term AG supplementation.

2. Oral administration of AG will increase serum endothelial NO concentration and reduce lipid peroxidation, indicating an improvement of endothelial function.

3. AG will reduce other cardiovascular risk factors such as hypertension.

4. The effect of AG on glycemia and blood pressure are associated with the enhancement of NO concentration.

3.2. Objectives

1. To observe the effects of AG on glycemic parameters (HbA1c, insulin and fasting blood glucose).

2. To determine whether AG effects the NO concentration by measuring serum nitrites/nitrates (NOx).

3. To observe the effect of AG on vasodilation indirectly by blood pressure measurements.

4. To determine whether AG effects lipid peroxidation by measuring serum malondialdehyde (MDA) using thiobarbituric acid (TBA) reaction.

5. To determine the correlation between NO and glycemic parameters, blood pressure, and lipid peroxidation.
CHAPTER FOUR

STUDY DESIGN AND METHODOLOGY
4. STUDY DESIGN AND METHODOLOGY

4.1. Subjects recruitment and profile

Subjects for the study were recruited through local newspaper advertisement and clinics in St. Michael's Hospital. Those who interested to participate in the study were invited to the Risk Factor Modification Centre at St. Michael's Hospital for an information session and initial screening. Out of 86 screened, 38 subjects who satisfied the study inclusion and exclusion criteria were recruited. All the eligible subjects signed a consent form approved by the St. Michael’s Hospital Research Ethics Committee (REB 97-016).

4.1.1. Inclusion criteria

1. Confirmed type 2 diabetes (≥ 1 year)
2. Age between 40 to 75 years
3. Glycosylated hemoglobin (HbA1c) between 6% and 9%
4. No change in diabetic medication (≥ 3 months) prior to the beginning of the study
5. Body mass index (BMI) less than 35 kg/m²
6. Clinically euthyroid
7. Normal renal and hepatic function
8. Willing to comply with study protocol and give informed consent

4.1.2. Exclusion criteria

1. Exogenous insulin or nitroglycerin user
2. Clinically significant diabetic complications
3. Recent (<6 months) stroke, myocardial infarction or unstable angina pectoris
4. Serious inflammation in the past three months

5. Taking ginseng or other herb with possible hypoglycemic effect

6. Heavy alcohol user (more than 3 drinks per day)

7. Cigarette smoker

Note: Patients who changed medication during the course of the study periods or during the washout period were excluded.

4.2. Preparation

4.2.1. Powder form of AG

The nutrient profile of the AG powder used in this study is shown in Table 4.1. The powder was made by grinding the roots which were taken from four years old *Panax quinquefolius* grown in Canada, and using regular extraction by a mixture of water and ethanol. The extract, commercially known as CNT 2000, was encapsulated in gelatin capsules, with each capsule containing 375mg AG, 75mg corn flour and 50mg aluminum oxide as the filter. All the AG capsules for the study were supplied by the manufacturer (Chai-Na-Ta Corp., B.C.), and obtained from the same manufacturer's batch.

Table 4.1. Nutrient profiles of American ginseng

<table>
<thead>
<tr>
<th></th>
<th>American ginseng (per 100g)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>352</td>
</tr>
<tr>
<td>Total carbohydrate (g)</td>
<td>67</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.8</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>24</td>
</tr>
</tbody>
</table>

* Analysis of samples provided by Chai-Na-Ta Corp. (IR Laboratories Inc., BC, 1997). Composition of ginsenosides profiles is not presented due to classified nature of this information.
4.2.2. Placebo

Placebo capsules were similar to AG capsules in appearance with the respect of colour, size and weight, containing 350mg corn flour and 50mg aluminum oxide.

4.3. Experiment design

The study employed a randomized, double-blind, placebo-controlled, crossover design. Prior to the beginning of the study, subjects had been followed for their medical information, diet and lifestyle for two months as a run-in period. The main purpose of this period was to assure that the subjects could achieve the best control of diabetes and stabilization of their condition on being given treatments. Right after the run-in period the subjects were assigned to receive either placebo or AG capsules along with their regular diabetic medication and diet for 8 weeks on the first phase of the study, after which they were switched to receive the alternative treatment following at least 4 weeks washout period. All the subjects were invited to the Risk Factor Modification Center at St. Michael’s Hospital every two weeks, after 12 to 14 hours fasting for tests during the whole course of the study. At the clinic, fasting blood was taken, anthropometry was measured and blood pressure was determined. A 3-day dietary record was submitted by each subject and checked by a dietitian. Upon each visit, subjects received a 14-day supply of the treatment capsules, at the same time they were advised to take 2 study capsules three times a day, 40 minutes before breakfast, lunch and dinner (Figure 4.1.)
Figure 4.1. Study schedule. ▲ Indicates fasting blood samples taken; blood pressure and anthropometry measurements; 3-day diet record submission; capsules dispense at week 0, 2, 4, 6; capsules return at week 2, 4, 6, 8.

4.4. Data collection and assays

4.4.1. Serum NOx

Serum NOx were measured by chemiluminescence method using NO Analyzer (NOA, Sievers 270b, Boulder, CO) at the Department of Medical Biophysics, the University of Western Ontario, London. Blood samples were centrifuged at 3000 rpm for 15 minutes at 4°C right after collection. The supernatant was removed and stored at -20°C. The nitric oxide assay was standardized by a calibration curve using known concentrations of nitrate (0.01 to 100 μmol/L) obtained from sodium nitrate. For each measurement, a 10μl sample was placed in a reducing vessel with 0.05mol/L of vanadium III chloride, 1mol/L of hydrochloric acid, and 100μl of antifoaming agent (Sievers) at 92°C. Each standard was analyzed three times, standard curves were
prepared with $R^2 > 0.999$. Samples were run in duplicate (triplicate if an obvious problem developed). The mean value was used for all subsequent analysis. A Chromatopak (Shimadzu, Japan) recorder was used to integrate the analyzer output signals.

4.4.2. Glycemic parameters

Fasting blood glucose was analyzed by glucose oxidase method with Vitros 950 Analyzer (Johnson & Johnson, USA) (Core Lab, St. Michael’s Hospital, Toronto).

HbA1c was measured by TOSOH Hemoglobin Analyzer (TOSOH, Japan) using HPLC method (Core Lab, St. Michael’s Hospital, Toronto).

Serum insulin concentration was determined by a double antibody radio-immunoassay method using commercially produced kits (Banting and Best Diabetes Centre Core Laboratory, the Toronto Hospital, Toronto).

4.4.3. Lipid peroxidation

Lipid peroxidation was estimated by measuring serum malondialdehyde (MDA) using thiobarbituric acid (TBA) reaction by HPLC method and reported as micromoles MDA per liter of serum (Department of Nutritional Sciences, University of Toronto).

4.4.4. Blood pressure

Specially designated and trained clinical staff measured blood pressure using a standard mercury Baum sphygmomanometer. After the patient had sat alone in a separated quiet examination room for at least 5 minutes, an appropriate cuff was applied to the left arm of the patient with the arm resting at heart level. Blood pressure was determined following American Heart Association’s recommendations. Systolic blood pressure was recorded as the highest pressure at which sounds are heard for at least two
consecutive heartbeats, and diastolic blood pressure was recorded at Korotkov phase 5, that is, when all sounds become inaudible. A minimum of two readings routinely, the final blood pressure were recorded as the mean of the two measurements. If difference between two measurements were more than 2 mmHg, a third measurement would be applied and the mean of three was used.

4.4.5. Anthropometry

Height, weight, waist and hip circumferences were obtained on each clinic visit using standard equipment and procedure. Body mass index (BMI) were calculated as body weight (kg) divided by square of height (m²).

4.4.6. Food records

Food intake was assessed from the three-day food records collected at week-1, week-0, week-2, week-4, week-6, and week-8. Energy and nutrients were calculated using ESHA Nutrition and Fitness Software (Version 6.11, Salem OR, USA).

4.4.7. Pill count

Mean daily pill count was assessed as the total number of pills dispensed minus the number of pills returned from each two weeks supply divided by 14 days. Any pills not taken but also not returned (verbally confirmed by subjects) were included in the total count.

4.5. Statistical analyses

All analyses were performed on the SAS statistical package (SAS for Windows, version 6.0, SAS Institute, Cary, NC). Results were expressed as mean±SEM unless indicated elsewhere. P<0.05 was considered to be statistically significant.
Pair t-test (two tailed) and repeated-measures analysis of variance (ANOVA) were used to assess interactive and independent effects of ginseng and placebo. General Linear Model (GLM) was used to compare the variables between groups after controlling for the confounding factors (sex, age, treatment sequence, and starting value). When data did not meet normality and equal variance, Wilcoxon Signed-Rank Test approximated with continuity correction was used to test the difference. Correlations were determined by using the Pearson correlation equation. The incidence of blood pressure in different treatments was compared by the Chi-Square test.
CHAPTER FIVE

RESULTS
5. RESULTS

There were 38 subjects who enrolled in the study. Only 24 of them completed the whole trial. Subjects' characteristics at enrollment are summarized in Table 5.1. Reasons for attrition of 14 subjects from the study included changing in diabetic medication (n=4), lacking of compliance to study protocol (n=5), developing acute illness (n=4) and personal reasons (n=1). Throughout the study periods all 24 subjects kept their medication unchanged. They consumed an ad libitum diet, maintained their body weight and regular life style.

5.1. Serum NOx

Serum NOx was determined three times at week 0, week 4 and week 8 during each study period (Table 5.2.). The absolute change difference between two treatments was 6.67±2.97 μmol/L, P=0.031 (Data did not meet normality and equal variance, Wilcoxon Signed-Rank Test approximated with continuity correction was used to test the difference). No statistical difference was found between week 0 and week 8 during placebo (-3.76±3.04μmol/L, P=0.23) or AG (2.91±1.95μmol/L, P=0.15) treatments (pair t-test, Figure 5.1.). The end difference between two treatments was 1.85±2.13μmol/L, P=0.28 (GLM ANOVA, adjusted by sex and starting value).
Table 5.1. Characteristics of 24 subjects with type 2 diabetes at baseline. 
Data are means±SD unless indicated

<table>
<thead>
<tr>
<th>n</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64 ± 7</td>
</tr>
<tr>
<td>Sex - M/F (n)</td>
<td>14/10</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79.7 ± 16</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.8 ± 4.6</td>
</tr>
<tr>
<td>Phase I randomization (Placebo/ AG)</td>
<td>10/14</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>6.1 ± 5</td>
</tr>
<tr>
<td>HbA₁C (%)</td>
<td>7.1 ± 1.2</td>
</tr>
<tr>
<td>Treatment of diabetes (n)</td>
<td></td>
</tr>
<tr>
<td>Diet only</td>
<td>8</td>
</tr>
<tr>
<td>Metformin</td>
<td>1</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>8</td>
</tr>
<tr>
<td>Sulfonylurea + Metformin</td>
<td>7</td>
</tr>
<tr>
<td>Subjects with anti-hypertensive treatment (n)</td>
<td></td>
</tr>
<tr>
<td>Ca²⁺ channel blockers</td>
<td>5</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>10</td>
</tr>
<tr>
<td>ARBs</td>
<td>2</td>
</tr>
<tr>
<td>β blockers</td>
<td>2</td>
</tr>
<tr>
<td>Diuretics</td>
<td>2</td>
</tr>
<tr>
<td>Combined treatment</td>
<td>5</td>
</tr>
<tr>
<td>Subjects with dietary supplements (no herbs) (n)</td>
<td></td>
</tr>
<tr>
<td>Vitamin E</td>
<td>6</td>
</tr>
<tr>
<td>Multivitamins</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 5.2. Serum nitrates/nitrites (NOx) concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (µmol/L)</td>
<td>24.46±3.16</td>
<td>17.79±1.85</td>
<td>20.71±2.58</td>
</tr>
<tr>
<td>American ginseng</td>
<td>19.65±1.72</td>
<td>19.25±2.51</td>
<td>22.56±2.18</td>
</tr>
</tbody>
</table>

Figure 5.1. Serum nitrates/nitrites (NOx) concentration changes over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM. AG: American ginseng. NOx: nitrates and nitrites. Wk-0: week 0. Absolute change difference = 6.67±2.97µmol/L, P=0.03 (Wilcoxon Signed-Rank Test).
5.2. Plasma HbA1c

Plasma HbA1c was analyzed at week 0 and week 8 of each study phase. The results are presented in Table 5.3. From week 0 to week 8, plasma HbA1c decreased 0.146±0.054%, P=0.043 (pair t-test) following AG treatment while during placebo it increased 0.038±0.056%, P=0.721 (pair t-test). More importantly, the end difference between two treatments was 0.288±0.101%, P=0.026 (GLM ANOVA, adjusted by sex and starting value). (Figure 5.2.).

5.3. Fasting blood glucose

Fasting blood glucose was measured every two weeks (Table 5.4.). It decreased 0.95±0.40 mmol/L (P=0.027, pair t-test) from week 0 to week 8 during AG treatment, while no significant change was found during placebo (0.54±0.27 mmol/L, P=0.056, pair t-test). The absolute change difference between two treatments was 1.49±0.45 mmol/L, P=0.003 (pair t-test). The end difference between two treatments was 0.71±0.30 mmol/L, P=0.088 (GLM ANOVA, adjusted by sex and starting value). In addition, fasting blood glucose decreased significantly from week 0 to week 2 following AG while it significantly increased from week 0 to week 6 during placebo (P<0.05, pair t-test). (Figure 5.3.)
Table 5.3. Plasma glycosylated hemoglobin (HbA1c) concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>week 0</th>
<th>week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (%)</td>
<td>7.263±0.220</td>
<td>7.283±0.214</td>
</tr>
<tr>
<td>American ginseng (%)</td>
<td>7.133±0.214</td>
<td>7.013±0.245*</td>
</tr>
</tbody>
</table>

* indicates significant difference from week 0, P<0.05 (pair t-test).
† indicates significant end difference from placebo at week 8.

Figure 5.2. Changes in plasma HbA1c concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM. AG: American ginseng. HbA1c: glycosylated hemoglobin. Wk-0: week 0.
Table 5.4. Fasting blood glucose concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Placebo (mmol/L)</th>
<th>American ginseng (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>8.38±0.48</td>
<td>9.16±0.58</td>
</tr>
<tr>
<td>Week 2</td>
<td>8.79±0.59</td>
<td>8.51±0.67</td>
</tr>
<tr>
<td>Week 4</td>
<td>8.74±0.51</td>
<td>8.74±0.52</td>
</tr>
<tr>
<td>Week 6</td>
<td>9.16±0.56</td>
<td>8.60±0.59</td>
</tr>
<tr>
<td>Week 8</td>
<td>8.92±0.47</td>
<td>8.21±0.55</td>
</tr>
</tbody>
</table>

* indicates significant difference from week 0, \( P<0.05 \) (pair t-test).

Figure 5.3. Fasting blood glucose concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM. AG: American ginseng. * indicates significant difference from week 0, \( P<0.05 \) (pair t-test). † indicates significant change difference between two treatments, \( P<0.05 \) (pair t-test).
5.4. Serum insulin

Serum insulin that increased over the course of AG treatment was clinically observable but not significantly different (9.46±7.47μmol/L, \( P=0.45 \)). Placebo treatment resulted in an insulin reduction. Neither this difference was significant (-11.97±12.92 μmol/L, \( P=0.89 \)). No significant change during the course of the study in either treatment, and no difference between two treatments at any time points were observed. Since data of serum insulin did not meet normality and equal variance, Wilcoxon Signed-Rank Test approximated with continuity correction was used to test the difference. (Table 5.5., Figure 5.4.)

5.5. Lipid peroxidation

Lipid peroxidation was determined in 23 subjects by measuring serum MDA at week 0 and week 8 of each study phase. The results are presented in Table 5.6. MDA decreased 0.14±0.07μmol/L, \( P=0.051 \)(pair t-test) following AG treatment from week 0 to week 8, while during placebo it increased 0.07±0.06μmol/L, \( P=0.315 \)(pair t-test). The absolute change difference between two treatments was 0.21±0.09μmol/L (\( P=0.03 \), pair t-test). (Figure 5.5.)
Table 5.5. Serum insulin concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>week 0</th>
<th>week 2</th>
<th>week 4</th>
<th>week 6</th>
<th>week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (μmol/L)</td>
<td>88.1±13.7</td>
<td>76.8±10.2</td>
<td>78.1±11.7</td>
<td>77.3±7.4</td>
<td>76.2±8.5</td>
</tr>
<tr>
<td>American ginseng (μmol/L)</td>
<td>77.8±8.7</td>
<td>74.1±9.1</td>
<td>74.0±8.9</td>
<td>85.2±13.5</td>
<td>87.3±10.9</td>
</tr>
</tbody>
</table>

Figure 5.4. Serum insulin concentration over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM. AG: American ginseng.
Table 5.6. Serum malondialdehyde concentration over 8 weeks treatment with American ginseng or placebo in 23 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>week 0</th>
<th>week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (µmol/L)</td>
<td>1.73±0.07</td>
<td>1.79±0.07</td>
</tr>
<tr>
<td>American ginseng (µmol/L)</td>
<td>1.86±0.08</td>
<td>1.72±0.05</td>
</tr>
</tbody>
</table>

Figure 5.5. Changes of serum malondialdehyde concentration over 8 weeks treatment with American ginseng or placebo in 23 subjects with type 2 diabetes. Data are means±SEM. MDA: malondialdehyde. AG: American ginseng. Wk-0: week 0. Absolute change difference=0.21±0.09µmol/L, P=0.03(pair t-test).
5.6. Systolic blood pressure (SBP)

During AG treatment, SBP decreased 11.0±2.0mmHg, P<0.001 (pair t-test) from week 0 to week 8, while no significant change occurred during placebo (0.3±2.1mmHg, P=0.91, pair t-test). The absolute change difference between two treatments was 11.5±2.2mmHg, P<0.001 (pair t-test). The end difference between two treatments was 5.6±2.7mmHg, P<0.001 (GLM ANOVA, adjusted by sex and starting value). (Table 5.7., Figure 5.6.)

5.7. Diastolic blood pressure (DBP)

DBP measured every two weeks during the study are presented in Table 5.9. Following AG treatment DBP decreased 5.3±1.5mmHg from week 0 to week 8, P=0.002 (pair t-test) while no significant change was found following placebo (-0.42±1.38mmHg, P=0.765, pair t-test). The absolute change difference between two treatments, in which DBP was lower with AG, was 4.8±2.2mmHg, P=0.035 (pair t-test). The end difference between two treatments was 2.5±1.4mmHg, P=0.123 (GLM ANOVA, adjusted by sex and starting value). (Figure 5.7.)
Table 5.7. Systolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>week 0</th>
<th>week 4</th>
<th>week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (mmHg)</td>
<td>131.3±4.0</td>
<td>128.6±4.1</td>
<td>131.7±3.8</td>
</tr>
<tr>
<td>American Ginseng (mmHg)</td>
<td>137.1±3.6</td>
<td>132.1±3.7</td>
<td>126.1±3.8*</td>
</tr>
</tbody>
</table>

* indicates significant difference from week 0, P<0.001 (pair t-test).

Figure 5.6. Systolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. AG: American ginseng. Data are means±SEM. BP: blood pressure. * indicates significant difference from week 0, P<0.001 (pair t-test). † indicates significant change difference between two treatments, P<0.001 (pair t-test). ‡ indicates significant end difference, P<0.001 (GLM ANOVA).
Table 5.8. Diastolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>week 0</th>
<th>week 4</th>
<th>week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (mmHg)</td>
<td>80.8±2.1</td>
<td>80.5±2.1</td>
<td>80.4±2.1</td>
</tr>
<tr>
<td>American ginseng (mmHg)</td>
<td>83.2±1.9</td>
<td>81.2±1.8</td>
<td>78.0±2.0</td>
</tr>
</tbody>
</table>

* indicates significant difference from week 0, P<0.05 (t-test).

Figure 5.7. Diastolic blood pressure over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM. AG: American ginseng. BP: blood pressure. * indicates significant difference from week 0, P<0.05 (pair t-test). † indicates significant change difference between two treatments, P<0.05 (pair t-test).
5.8. Blood pressure reclassification

Following AG treatment, eight subjects reclassified from hypertension (n=7) and high-normal (n=1) BP category to the normal BP category (Table 5.9., according to the criteria from the Sixth Report of the Joint National Committee on Prevention, Evaluation and Treatment of High Blood Pressure). However in placebo group, number of normal BP category subjects stayed unchanged (n=10), one subject increased BP from high normal to hypertension category (P<0.005, $\chi^2$ test). (Figure 5.8., 5.9.)

<table>
<thead>
<tr>
<th>Table 5.9. Number of subjects with type 2 diabetes in different blood pressure category at week 0 and week 8 with treatment of American ginseng or placebo.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Placebo</td>
</tr>
<tr>
<td>week 0 (n)</td>
</tr>
<tr>
<td>week 8 (n)</td>
</tr>
<tr>
<td>American ginseng</td>
</tr>
<tr>
<td>week 0 (n)</td>
</tr>
<tr>
<td>week 8 (n)</td>
</tr>
</tbody>
</table>
**Figure 5.8.** Number of subjects with type 2 diabetes in different blood pressure category at week 0 and week 8 with treatment of American ginseng. AG: American ginseng. BP: blood pressure. Wk-0: week 0, Wk-8: week 8

**Figure 5.9** Number of subjects with type 2 diabetes in different blood pressure category at week 0 and week 8 with treatment of placebo. AG: American ginseng. BP: blood pressure. Wk-0: week 0, Wk-8: week 8
5.9. Body Weight

Subjects were asked to maintain their body weight throughout the study. Changes in body weight during placebo and AG treatments are presented in Table 5.10. There was no significant change in body weight during the course of the study in either treatments. Also, no difference between two treatments at the end of the study was found. (P>0.05, paired t-test and GLM ANOVA), (Figure 5.10).

5.10. Food record and pill count

Mean dietary intake and pill count during placebo and AG treatments in 24 type 2 diabetic subjects are presented in Table 5.11.

Daily intake represented the mean of three days food records collected at week 0, week 2, week 4, week 6, and week 8 on each study phase. Energy and nutrients content were calculated by ESHA Nutrition and Fitness Software (version 6.11, Salem OR, USA). There was no significant difference in energy and nutrient intake between two treatments during the study period (P>0.05, paired t-test).

Mean daily pill count was assessed as the total number of pills dispensed minus the number of returned from each two weeks supply divided by 14 days. Subjects were advised to take 2 capsules three times a day, 40 minutes before breakfast, lunch and dinner. The major reason for skipping pills was subjects eating outside. However, there was no difference in pills consumption between two treatments (P>0.05, paired t-test).
Table 5.10. Body weight over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM.

<table>
<thead>
<tr>
<th></th>
<th>week 0</th>
<th>week 2</th>
<th>week 4</th>
<th>week 6</th>
<th>week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (kg)</td>
<td>79.7±3.4</td>
<td>79.5±3.4</td>
<td>79.6±3.4</td>
<td>79.4±3.4</td>
<td>79.5±3.2</td>
</tr>
<tr>
<td>American ginseng (kg)</td>
<td>79.7±3.3</td>
<td>79.4±3.2</td>
<td>79.5±3.2</td>
<td>79.4±3.2</td>
<td>79.1±3.1</td>
</tr>
</tbody>
</table>

Figure 5.10. Body weight over 8 weeks treatment with American ginseng or placebo in 24 subjects with type 2 diabetes. Data are means±SEM. AG: American ginseng.
Table 5.11. Average daily intake of energy and macro nutrients over study period in placebo or American ginseng treatment

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Placebo</th>
<th>Ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>1769 ± 102</td>
<td>1753 ± 78</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>19.6±1.1</td>
<td>19.6±1.1</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>51.3±3.3</td>
<td>49.6±3.0</td>
</tr>
<tr>
<td>Total fat (%)</td>
<td>28.8±2.3</td>
<td>31±2.1</td>
</tr>
<tr>
<td>SFA (%)</td>
<td>9.4±1.0</td>
<td>9.9±0.8</td>
</tr>
<tr>
<td>PUFA (%)</td>
<td>5.1±0.4</td>
<td>6.1±0.6</td>
</tr>
<tr>
<td>MUFA (%)</td>
<td>10±0.9</td>
<td>11.2±0.9</td>
</tr>
<tr>
<td>Total fiber (g)</td>
<td>25.0 ± 2.0</td>
<td>22.8 ± 1.9</td>
</tr>
<tr>
<td>Soluble fiber (g)</td>
<td>6.1 ± 2.6</td>
<td>5.8 ± 2.6</td>
</tr>
<tr>
<td>Pill count (capsules/day)</td>
<td>5.47±0.14</td>
<td>5.45±0.15</td>
</tr>
<tr>
<td>Quantity of American ginseng (g/day)</td>
<td>0</td>
<td>1.91±0.52</td>
</tr>
</tbody>
</table>

1 Means±SEM of 3-day food records on each study phase. SFA: Saturated fatty acid. PUFA: Polyunsaturated fatty acid. MUFA: Monounsaturated fatty acid.
2 Including trans fatty acids.
5.11. Correlation between serum NOx and other parameters

Pearson correlation was performed to compare

(1) serum NOx and other parameters at week 0;

(2) absolute change of NOx and other parameters during the study course in both treatments;

(3) percent change of NOx and other parameters during the study course in both treatments.

The only significant finding is that the percent end difference of NOx was negative correlated to HbA1c (R= -0.42, P=0.043) (Figure 5.11.).

5.12. Effect according to subjective symptoms

Two subjects, one in placebo and one in AG group, discontinued taking supplements due to abdominal discomfort and diarrhea. No other symptoms or side effects were observed.
Figure 5.11. Correlation between the percent end difference of NOx and HbA1c. 
% end difference = (end value in American ginseng treatment – end value in placebo treatment)/end value in placebo treatment*100. NOx: nitrates and nitrites. HbA1c: glycosylated hemoglobin.
CHAPTER SIX

DISCUSSION AND CONCLUSIONS
6. DISCUSSION AND CONCLUSIONS

6.1. Discussion

The results of this study indicated that in type 2 diabetic subjects oral administration of AG significantly lowered HbA1c (Figure 5.2.), reduced fasting blood glucose (Figure 5.3.), and improved blood pressure (Figure 5.6, 5.7). The hypoglycemic effect of AG seems to be correlated with increased endothelial NO level (Figure 5.11.). Changing in serum NO concentration may indicate an improvement in endothelial function, which could be explained, at least partially, by the reduced endothelium damage due to lower glucose concentration and less lipid peroxidation (Figure 5.5). Furthermore, the vasodilation effect of AG could be elucidated by the increase of NO level(Figure 5.1).

6.1.1. Ginseng and glycemic control: the role of NO

In this study, the hypoglycemic effect of AG in type 2 diabetes was confirmed by significant decreases in HbA1c (Table 5.3, Figure 5.2.) and fasting glucose (Table 5.4, Figure 5.3.), which are concord with other clinical and animal study data. The mechanism of AG effect on glycemic regulation is not clear at present. Animal data support several possibilities that ginseng modulate digestion, insulin synthesis, insulin release, insulin degradation, insulin receptor sensitivity, glucose transport systems and their combination. Based on current study result, the role of NO in hypoglycemic effect of AG will be discussed.
6.1.1.1. Modulation of digestion

There is indirect evidence suggesting that ginseng may affect the rate of digestion. An inhibition of neuronal discharge frequency from the gastric compartment of the brain stem in rats by AG has been observed (Yuan et al, 1998). Inhibition of gastric secretion by Chinese ginseng has also been observed in rats (Suzuki et al, 1991). The result of both indicated ginseng slowed the digestion of food, decreasing the rate of carbohydrate absorption into portal hepatic circulation.

However, this hypothesis does not appear to be supported by the blood glucose profiles in our acute AG studies in which we did not observe lower values in the first 30 minutes in type 2 diabetes: the absorptive phase of the glucose curve (Vuksan et al, 2000a; Vuksan et al, 2000b). Both soluble dietary fiber and acarbose (Jenkins et al, 1979; Wolfever, 1998), which operate through delaying or inhibiting the absorption of carbohydrate in the gut, exhibited a flat blood glucose concentration throughout postprandial response. In the case of AG, reductions were observed 30 minutes after glucose challenge right following the glucose peak rise: the disposal phase of the glucose curve. Stronger support therefore, is offered for a ginseng modulating effect on insulin secretion or sensitivity rather than modulating digestion per se. There is no evidence that demonstrated NO has any relationship with food digestion.

6.1.1.2. Modulation of insulin sensitivity

A direct effect of ginseng on glucose transporter system has been shown in mice and cell lines. Chinese ginseng was observed to increase GLUT 2 protein in the livers of normal and hyperglycemic mice (Ohnishi et al, 1996). Its saponins, especially Rb1,
which is comparatively higher in AG than Chinese one, were also noticed to increase glucose uptake into sheep erythrocytes (GLUT 2) in a dose dependent manner (Hasegawa et al, 1994).

This hypothesis may be expanded to include a mediating role of NO. Several studies have indicated that the antioxidant and organ-protective actions of ginseng may be linked to enhanced NO synthesis (Gillis, 1998). It was recently shown that insulin stimulated glucose uptake in rat skeletal muscles and adipose tissue, which are transported by GLUT4, is NO dependent (Roy et al, 1998). Enhanced NO synthesis by ginseng in the endothelium of lung, heart, kidney and corpus cavernosum has been noticed in animal models (Gillis, 1998).

The current study results demonstrated that after 8 weeks AG treatment, glycemic control improved by reduced HbA1c and fasting blood glucose. In the mean time, serum NOx, the stable metabolites of NO, is significantly higher than that after the placebo treatment (Figure 5.2). In addition, a significantly negative correlation was found between the change of NO and HbA1c. It is possible that improvement to glycemic control reversed endothelial dysfunction in type 2 diabetes and therefore, increased endothelial dependent NO generation. Or vice versa, endothelial derived NO may activate more glucose transporter or insulin receptors in the organs, stimulating glucose uptake and decreasing blood glucose level. Alternatively, a mediating role of cholinergic stimulation or adrenergic blockade may be involved. Rb1, Rg1, and Re were noticed to increase choline acetyltransferase mRNA, protein, and activity in rat brains, suggesting possible enhancement of acetylcholine secretion (Yamaguchi et al, 1997; Salim et al, 1997). Several studies indicated insulin resistance was caused by hepatic cholinergic
interruption and could be reversed by acetylcholine administration (Lekas et al, 1999; Xie and Lautt, 1996). As demonstrated in Fugure 2.5, acetylcholine is an agent in blood which activates the NO synthesis from L-arginine. The mechanism of acetylcholine action on insulin sensitivity is likely to have NO involved as a signal.

6.1.1.3. Modulation of insulin secretion

Some ginseng fractions have also been noticed to affect insulin release directly. DPG-3-2, a component of ginseng prepared from water extraction was shown to stimulate insulin biosynthesis in different preparations of mice islets and rat pancreas (Waki et al, 1982). Similarly, various fractions of ginseng were reported to increase glucose stimulated insulin secretion in alloxan diabetic mice at potency similar to sulfonylureases (Kimura et al, 1981).

Again NO might be playing an important role. In pancreatic islets, NO produced on exposure to cytokines mediates β-cell injury leading to diabetes mellitus. NO released in response to exposure of pancreatic islets to cytokines may be formed either in the β-cell themselves, leading to self-destruction, or in intra-islet macrophages and endothelial cells from which it diffuses in to β-cells (Spinas, 1999). On the other hand, L-arginine-derived NO may participate in the signal transduction pathway of physiological insulin secretion (Spinas, 1999). The presence of NOS in the secretory granules of α- and δ-cells suggests that NO is produced in these cells and subsequently diffuses to β-cells and exert a paracrine effect. The stimulatory effect of NO on insulin secretion is subtle and pertains mainly to the early phase of glucose-stimulated insulin release. The mechanism by which NO triggers insulin release remains to be elucidated. One putative mechanism
could be that NO exerts its action via increasing intracellular calcium through mobilization of calcium from intracellular pools such as the endoplasmic reticulum or from mitochondria (Laffranchi et al, 1995). Besides, NO may target the insulin vesicle and modulated synaptic vesicle docking fusion reactions, as has been observed in synaptosomes, or act via cell surface receptors or ion channels of the β-cells (Spinas, 1999).

In Sotaniemi’s study, fasting insulin had no significant change with ginseng treatment (Sotaniemi et al, 1995). Neither our study was able to find significant different between AG and placebo, although following AG treatment, serum insulin increased 9.45±7.46μM, while during placebo treatment there was a reduction of 12.0±12.9μM. It is notable that in both studies, the standard deviation of insulin was high. This may be due to the wide range in subjects’ duration of diabetes, and the different medication/dosage they were using. Also we failed to see the significant correlation in change difference between NOx and insulin (R=0.03, P=0.872, Pearson correlation test). Therefore the improved glycemic control as demonstrated by these clinical studies may count more on the improvement of insulin sensitivity rather than insulin synthesis or release from pancreas.

6.1.2. Ginseng and blood pressure: the role of NO

Elevated BP is known to be a major risk factor for all cause mortality and diabetes related complications in subjects with type 2 diabetes. This has been demonstrated in two recent large prospective studies, HOT and UKPDS (Hansson et al, 1998; UKPDS, 1998). These studies also revealed that adequate BP control not only reduced markedly
microvascular complications, but also overall cardiovascular mortality. The effect was beyond the tight glycemic control per se. Between 38 and 66% of newly diagnosed type 2 diabetic patients have hypertension. The UKPDS study emphasized that reducing BP has the highest priority in type 2 diabetes care (UKPDS, 1998; Uusitupa et al., 1993).

With the respect to ginseng, it has been found that intravenous administration of ginsenosides lowered blood pressure in a dose-dependent manner in anesthetized rats (Kim et al, 1994). In human, after 8 weeks of Red Korean ginseng administration, a significant decline in SBP was noticed from the essential hypertensive patient in contrast with the white coat hypertensive patients (Han et al, 1997). The decline in DBP was also regarded as a string of the beneficial pharmacological reaction of ginseng (Han et al, 1997).

In the current study, both the SBP and DBP significantly decreased at the end of Wk-8 after AG treatment while there was no change in the placebo group. SBP also showed a significant end difference between two treatments (Figure 5.6). More importantly, according to the criteria from the Sixth Report of the Joint National Committee On Prevention, Detection, Evaluation and Treatment of High Blood Pressure (National Institutes of Health, 1997), there were eight subjects improved their blood pressure from hypertensive (>140/90mmHg) or high-normal (130-139/85-89mmHg) category to normal BP category (<130/85mmHg) during AG treatment, while none improved during placebo. It is essential that in patients with diabetes, antihypertensive drug therapy should be initiated along with lifestyle modifications to reduce arterial BP to below 130/85 mmHg. Patients with diabetes could experience a similar or greater
reduction of coronary heart diseases and total cardiovascular events compared with persons without diabetes following BP reduction (National Institutes of Health, 1997).

The effects of ginseng biochemical and pharmacological effect are being gradually disclosed. Ginseng's influence on blood pressure, however, is still a subject of controversy. Reports on the antihypertensive effect of ginseng as a result of clinical trial are still rare. The mechanism of the antihypertensive effect of ginseng, although ambiguously, was reported to enhance the release of NO, either directly or indirectly through insulin action (Han et al, 1998).

6.1.2.1. Direct modulation of NO generation

In the current study we measured plasma NOx at the same time points of measuring blood pressure. As per animal study data, it was not surprised to find blood pressure reduced while NOx increase in AG treatment group. Animal studies suggest that ginsenosides may decrease blood pressure by stimulating the release of NO, which relaxes blood vessels by enhancing the production of cyclic GMP (Chen, 1996). This idea is supported by the fact that ginsenosides relaxed rings of both rat and rabbit aorta in the presence of endothelium but affected only minimally those without. An endothelium-dependent vasodilator response to ginsenosides has also been demonstrated in the perfused pulmonary bed of the rabbit (Kim et al., 1992). Furthermore, the endothelium-dependent relaxation evoked by ginsenosides was reduced by L-NMMA, an inhibitor of NOS. Thus, these observations indicated that ginsenosides enhances the production of NO from L-arginine in the vascular endothelium (Kim et al, 1994).
Our study results indicated that in type 2 diabetic patients, serum NOx increased after 8 weeks of AG administration in contrast to placebo. As a signaling gas, NO generated from endothelium plays an important role in vasodilation. Consequently, the decrease of blood pressure both in systolic and diastolic in our study subjects was likely resulted from the increase of NO generation due to its vasodialation action. However, we were not able to find statistically significant correlation between NOx and blood pressure. The main reason for this could be due to relatively small sample size, and large NOx variability in the baseline value and in responses to AG or placebo treatments.

6.1.2.2. Modulation of NO generation through insulin action

Hypertension is associated with insulin resistant states, but the mechanisms linking hypertension with insulin resistance, which is the decreased sensitivity to insulin with respect to glucose uptake and metabolism, are not well understood. Since insulin resistance usually leads to compensatory hyperinsulinemia, some investigators have hypothesized a direct role for insulin in hypertension (Reaven, 1995; Baron, 1994). The effects of insulin to promote renal tubular reabsorption of sodium, sympathetic nervous system activity, and proliferation of vascular smooth muscle cells tend to increase plasma volume, cardiac output, and peripheral vascular resistance. However, these effects are opposed by direct vasodilatory actions of insulin in some vascular beds. Thus, the net hemodynamic effect of insulin, if any, is a tendency to lower blood pressure. Interestingly, drugs that improve insulin sensitivity also lower blood pressure in hypertensive humans and rats (Ogihara et al., 1995; Lee et al., 1994). This suggests that
it may be abnormalities underlying insulin resistance rather than insulin per se that are causally related to hypertension (Zeng and Quon, 1996)

An elegant series of experiments by Baron and co-workers showed that sensitivity to the vasodilatory action of insulin is positively correlated with insulin sensitivity with respect to glucose uptake in normal, obese, and diabetic individuals. Furthermore, using inhibitors of NOS, they have shown that the vasodilatory action of insulin is most likely mediated by NO (Steinberg et al. 1994). Thus, it is possible that defects in insulin signaling leading to insulin resistance with respect to glucose metabolism may also lead to defects in insulin-stimulated production of NO. This would be predicted to cause an impaired vasodilatory response to insulin resulting in a relative elevation in peripheral vascular resistance that may contribute to hypertension.

Ginseng fractions have been noticed to affect insulin biosynthesis in different preparations of mice islets and rat pancreas (Waki et al. 1982). In addition, various fractions of ginseng were reported to increase glucose stimulated insulin secretion in alloxan diabetic mice at potency similar to sulfonylureases (Kimura et al., 1981). In the current study we demonstrated that with AG treatment, plasma HbAlc decreased significantly from Wk-0 to Wk-8, which was correlated to the significant increase in serum NOx, while blood pressure decreased simultaneously. However, we were not able to find statistically significant correlation between insulin to either NOx or blood pressure level. This may systematically due to high variability of serum insulin level and relatively small sample size, or possibly further indicted that the hypoglycemic function of ginseng focused more on modulating insulin sensitivity.
6.1.2.3. Modulation of NO level through less lipid peroxidation

Peroxidation of lipids exposed to oxygen is responsible for damage to tissues, where it may be a cause of cancer, inflammatory diseases, atherosclerosis, aging, etc. The deleterious effects are considered to be caused by free radicals produced during peroxide formation from polyunsaturated fatty acids. Free radicals such as superoxide is a potent scavenger of NO (Figure 2.5.). Antioxidant action of ginseng had been demonstrated by several animal studies. In the current study, lipid peroxidation was reduced after long term AG treatment while the serum NOx was increased at the mean time. It is possible that AG declined oxidative stress, and therefore reduced degradation of NO.

6.1.3. Summary

Although the hypoglycemic and antihypertensive action of AG is still elusive, based on animal studies and the current study observation, we proposed several possible mechanisms of AG effects, in which NO may play an important role:

1. Hypoglycemic effect of AG may partially reverse the endothelial dysfunction in type 2 diabetes, therefore increase endothelial dependent NO generation.

2. AG could directly improve endothelium function, stimulate NO synthesis in vascular endothelial cells and therefore, dilate all types of blood vessels, which results in a decrease of blood pressure.

3. Less lipid peroxidation indicated antioxidant action of AG, which protects endothelial function from free radical injury in type 2 diabetes and reduced NO degradation.
6.2. Conclusions

1. AG improved glycemic control in type 2 diabetes by reducing fasting blood glucose and plasma HbA1c.

2. Serum NOx had a significant increase following long term AG treatment, which indicated the improvement of endothelial function in type 2 diabetes.

3. AG reduced cardiovascular risk factors in type 2 diabetes by decreasing systolic and diastolic blood pressure.

4. AG significantly reduced lipid peroxidation in type 2 diabetes, indicating its antioxidant potential.

5. Increased NOx was correlated to the decrease of plasma HbA1c, indicating the glycemic control improvement is associated with NO concentration and endothelial function.

6.3. Directions for future research

Despite the interesting learning from the current study, it is still questionable whether ginseng has a long-term beneficial effect on hyperglycemia and hypertension in type 2 diabetes. Besides, the association between ginseng and the improvement of endothelial function, and the role of NO generation should be further investigated.

1. To investigate the effect of AG on NO generation, serum NOx values should be determined in non-diabetic subjects in a similar study design.
2. We investigated one dose of AG oral administration for 8 weeks in the current study. An implication for future studies may include different dose of ginseng supplementation.

3. The ginseng used in this study is a standardized AG extract. To verify the possible mechanism and active components of ginseng that are accountable for its effects, individual or a group of ginsenosides should be tested in the future studies.

4. Subjects in this study were not controlled for their food intake. Possibly, the food consumed 2-3 days prior to blood sample collection may effect the serum NOx concentration. Standardization of food intake especially with respect to high nitrates or nitrites containing products before each clinic visit should be controlled in the future studies.

5. High variability in insulin concentration may be caused by different diabetes duration of subjects. Hyperinsulinemia is the characteristic of subjects with IGT or short diabetes history. Insulin level and sensitivity may vary considerably within type 2 diabetic subjects. It will be interesting to compare the ginseng effect on non-diabetic, subjects with IGT, subjects with longer history of type 2 diabetes who have low insulin secretion, and subjects with type 1 diabetes.
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8. APPENDIX
APPENDIX I

CONSENT FORM

USE OF NORTH AMERICAN GINSENG IN
THE MANAGEMENT OF TYPE 2 DIABETES

INTRODUCTION

Ginseng is a man-shaped root indigenous to Asia, North America and Russia. Its value in promoting overall body health has long been recognized by the Chinese as precious and renowned. In fact, they have been using ginseng for more than 5,000 years. Ginseng has traditionally been thought to prolong life, boost energy, elevate sex drive and soothe base emotions. Over the years, many other conditions, including diabetes, have been reported to be alleviated by the constant consumption of ginseng. However, these claims are generally based upon anecdotal evidence. Therefore, the purpose of this study is to investigate the efficacy of North American ginseng in type 2 diabetes.

PROCEDURES

Before entering study, I will have a medical history, blood and urine tests, blood pressure measurement, and dietary record analysis.

This study will last for 5-6 months consisting of two 8-week treatment periods separated by a 4-week washout period in which I shall receive neither ginseng products, placebo diet supplements.

If I participate in this study, I will be asked to consume ginseng or placebo capsules for a period of 8 weeks. I will visit the Clinical Nutrition and Risk Factor Modification Centre, 61 Queen Street East for 12 mornings after a 10-12 hour-overnight fast, namely, at one week before each phase starts, week 0, week 2, week 4, week 6, and week 8. Each of these visits will take approximately one and a half hours in which my body weight, blood pressure, and blood circulation will be measured and recorded. I will submit my quality of life questionnaire, 3-day dietary record and 24-hour urine collection. In the mean time, I will give 30mL or 6 teaspoon of venous blood.
POTENTIAL HARMs (INJURY, DISCOMFORTS, OR INCONVENIENCE)

I understand that there are no known risks from ginseng powder and extract. However, I may experience discomfort and bruising from the venous blood collection. Bruising may occur when the needle is removed but this can be prevent by keeping pressure on the site for 3-5 minutes after the removal of the needle. If bruising occurs, it will go away in 2-3 days.

BENEFITS

I may expect to benefit from this study in the following ways: my blood sugar, blood lipids, blood pressure and blood circulation may improve. I will receive the benefits of evaluation of symptoms and general health discussions with the doctor and help in finding additional treatment if needed. I will have a chance to contribute to a study which may be of benefit to people with type 2 diabetes in the future.

CONFIDENTIALITY

I understand that I shall be informed of the results of this study and that these results will be confidential and shown to no one without my expressed consent unless required by law. If I desire, my results may be forwarded to family physician.

PUBLICATION OF RESULTS

The results from this study may be presented at public forums such as conferences or seminars. Publication of these results may also occur in scientific, lay, or promotional media. In either case I understand that I will not be identified by name. Instead only data for the group as a whole will be reported or where it is necessary to report individual data, non-traceable identification numbers will be used.

PARTICIPATION AND WITHDRAWL

I understand that the study investigator may stop my being in the study at any time without my consent. My participation may be discontinued if the study investigator judges that this is in my best interest or if I fail to comply to study procedures.

QUALIFICATIONS

I understand I cannot be in this study if I abuse alcohol or drugs, or if I have a serious illness that is not under control. I am above the age of 18 years.

CONSENT

I acknowledge that I have been given sufficient time to read this consent form. The research described there-in has been explained adequately, and any questions that I had
have been answered to my satisfaction. I understand that I may reach Dr. Vladimir Vuksan at (416) 867-7450 if I have further questions pertaining to the study. I freely volunteer to participate in this study. I may quit at any time by notifying the study investigator. By agreeing to participate, I understand that I have not waived my legal rights nor released the investigator, sponsors, or involved institutions from their legal and professional duties.

I hereby consent to participate and have been given a copy of this consent form.

Volunteer's
Name: ..............................................................................................................................

Volunteer's
Signature:.......................... Date: .................................................................

Investigator's
Name: .......................................................... Date: ........................................................

Investigator's
Signature:.......................... Date: .................................................................

Witness
Name: .................................................. Date: ........................................................

Witness
Signature:........................................ Date: ........................................................

Volunteer's Name: .......................................................... Date: ........................................................