METABOLIC EFFECTS OF COMBINING
AMERICAN GINSENG (Panax quinquefolius L.)
AND VISCOUS FIBRE IN THE TREATMENT OF
TYPE 2 DIABETES

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Abstract

The aim of the thesis was to determine whether synergistic or additive metabolic benefits could be derived with the use of two plant-based components with independent physiological activities and whether the use of these two agents would improve diabetes control in the long term.

Viscous fibres reduce postprandial glycaemia, insulinaemia and gastrointestinal hormones levels, their effectiveness being dependent on the viscosity of the fibre. Long term studies with viscous fibre have shown reductions in serum cholesterol, and improvements in metabolic control and blood pressure in type 2 diabetes. Konjac mannan fibre (KJM) is a high viscosity, viscous fibre. American ginseng (AG) increases insulin secretion and improves glycaemic control in type 2 diabetes. The suggested active ginseng components are the ginsenosides. As both AG and KJM have been shown to improve diabetes control through independent physiological mechanisms, this thesis explores the possible benefits of using them in combination.

To test potential additive effects of KJM and AG, an acute experiment was performed in thirteen subjects with type 2 diabetes where AG and/or KJM were incorporated into a liquid meal replacement as a breakfast, followed 4 hours later by a standard lunch. Contrary to previous findings, neither AG or KJM when given individually, reduced postprandial glucose and insulin levels by the expected amounts; moreover the combination did not result in glucose levels significantly different from control. The study did demonstrate that KJM flattens the postprandial response of glucose-dependent insulinoieptide (p<0.04) and glucagon like peptide-1 (p<0.02). In addition KJM reduced the glucose area under the curve to a standard meal following the fibre enriched breakfast. Subsequent experiments showed that, despite high ginsenoside levels, the AG used was ineffective and the liquid replacement meal inhibited the gel formation of the KJM.

In preparation for a long-term intervention study, acute experiments were performed to select a physiologically effective AG, and to determine the most effective and palatable method of KJM incorporation. Incorporation of KJM into bread, capsules
and margarine reduced the incremental areas under the glucose curve by 3%, 12% and 34% compared with control, with only the margarine being significantly different (p<0.001). Further experiments showed that incorporation of 1g of KJM into margarine and 4g of KJM into bread reduced the glucose AUC to a similar extent. KJM capsules has no acute effect on postprandial glycaemia, regardless of the timing of their administration relative to the meal.

Three months of 6g of viscous fibre and 3g of AG administration to 30 subjects with type 2 diabetes resulted in significant percent-change difference from control in HbA1c (-4.1±1.4%, p<0.01), total cholesterol (-5.7±1.9%, p<0.00), LDL-cholesterol (-8.2±3.1%, p<0.002) and apoliporotein B (-9.0±2.3%, p<0.0005). It was estimated the combination of viscous fibre and AG resulted in a 14% risk reduction in coronary heart disease.

The studies in this thesis illustrate some of the difficulties of working with “natural” products but also their potentially effective use in the treatment of type 2 diabetes.
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Chapter 1

LITERATURE REVIEW

1.1 General Introduction

The use of complementary and alternative medicine (CAM) is increasing worldwide. Despite scepticism and lack of scientific data it has been estimated that the use of CAM has increased by 68% from 1990-1997 in the US (Eisenberg et al., 1998). As a result there has been a call for randomised clinical trials to evaluate the safety and efficacy of CAM and provide a basis for legitimate health claims (Angell and Kassirer, 1998; Ernst et al., 2002). Evidence regarding the health claims for the use of CAM is emerging slowly. In a systematic review of the use of herbs and dietary supplements on glycaemic control by Yeh and colleagues (2003) they concluded that, in general, there is still insufficient evidence to draw definite conclusions. However out of the 36 herb and 9 vitamin and mineral supplements reviewed they identified **Coccinia indica** (Ivy gourd) and American ginseng as having the best evidence for efficacy supported by adequately designed randomised controlled trials (Yeh et al., 2003).

American ginseng has been of interest to our research group as it has been shown to improve long term diabetic control (Sotaniemi et al., 1995). Our group has undertaken a series of acute studies to investigate the effects of ginseng and have shown that American ginseng lowers postprandial glycaemia in both healthy individuals and those with type 2 diabetes (Vuksan et al., 2000a). A subsequent long-term study showed that ginseng improved diabetic control (Vuksan et al., 2001b). Postulated mechanisms of action of ginseng include: increase of insulin-stimulated glucose uptake (Spinias et al., 1998), increase of insulin secretion (Yokozawa et al., 1985b) and delayed gastric emptying (Onumra et al., 1999). Prior to the interest in ginseng our research group had studied the viscous fibre, konjac mannan, and had shown, both in short and long term studies, that konjac mannan fibre improves metabolic control in individuals with the insulin resistance syndrome and type 2
diabetes. The postulated mechanism of action for viscous fibre is that it delays glucose absorption and so attenuates the postprandial glucose and insulin responses.

Type 2 diabetes is now commonly treated with two or more oral hypoglycaemic medications, which employ different mechanisms of action to treat diabetes. Combinations have been found to be more effective than mono-therapy; an example of such a combination being sulfonylurea taken with metformin. As ginseng and konjac mannan both employ different mechanisms of action to improve diabetes control, the next logical step was to explore the treatment potential of these two compounds used in combination in the treatment of type 2 diabetes.

The following review of the literature will summarise the incidence, aetiology, and pathophysiology of type 2 diabetes and the role of insulin, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) in diabetes. In addition the current literature on American ginseng and konjac mannan fibre will be reviewed with special emphasis on their possible role in the treatment of diabetes.

1.2. Type 2 Diabetes

1.2.1 Incidence

Diabetes is a major cause of morbidity and mortality in the US (Harris, 1998) with an estimated health burden of $98 billion (ADA, 1998). Over the last ten years a dramatic increase of 33% in the incidence of diabetes has been observed in the US (Mokdad et al., 2003). Currently at least 16 million Americans have diabetes and this figure is expected to rise to 22 million by 2025 (Mokdad et al., 2003) and to 29 million in 2050 (Boyle et al., 2001). Similarly in Canada there are at present about 1.5 million people with diabetes (5.8% of the population) (Health Canada, 1999), and 3-5% of the general adult population is thought to have undiagnosed type 2 diabetes (Tan and MacLean, 1995; Williams and Wild, 2003). Figures for the UK are more difficult to obtain (Williams and Wild, 2003), the WHO estimated the prevalence to be 2.7%, but these data were derived from a Dutch study (Mooy et al., 2000). Globally the figures are alarming too, it has been estimated that the incidence of
diabetes will increase from 135 million in 1995 to 300 million in 2025 (King et al., 1998). The most recent data from the Centres for Disease Control Behavioral Risk Factor Surveillance System show that the greatest increase (70%) came in the younger adult cohorts aged 30-39y (Mokdad et al., 2003). The incidence data for insulin resistance are also alarming. In the Botnia Study cohort of Finnish and Swedish adults, using the euglycaemic-hyperinsulinaemic clamp, it was shown that insulin resistance occurred in 10% of men and 15% of women with normal glucose tolerance, 42 and 64% of those with IFT/IGT and 78 and 84% of those with type 2 diabetes respectively (Isomaa et al., 2001). Similar results were obtained using the Homeostasis Model Assessment (HOMA) in both the Bruneck Study cohort (Bonora et al., 1998) and the San Antonio Heart Study (Haffner et al., 2000) in which insulin resistance was observed in 84% and 82.4% respectively in those with type 2 diabetes. Estimates suggest that the prevalence of insulin resistance would be >30% in Canadian adults (Ekoe, 1999). Therefore strategies for the prevention of the development of insulin resistance and ultimately diabetes are urgently needed.

1.2.2 Pathophysiology

Type 2 diabetes represents a heterogeneous group of metabolic disorders resulting in hyperglycaemia. In the San Antonio Heart Study it was estimated that those who converted to diabetes over the seven year period had significantly higher body mass index, waist circumference, triacylglycerol (TAG) concentration, and blood pressure and lower HDL cholesterol levels than those who did not convert to diabetes (Haffner et al., 2000). The distribution by metabolic status among converters to type 2 diabetes were as follows: 54% had both insulin resistance and low insulin secretion, 28% were insulin resistant only, 15.9% had low insulin secretion only, and 1.5% were neither insulin resistant or had reduced insulin secretion (Haffner et al., 2000). Therefore over 80% of those who developed diabetes over the seven years of the study had prior insulin resistance. This fits in well with the postulated sequence for development of diabetes which suggests that the earliest detectable abnormality in type 2 diabetes is an impairment of the body to respond to insulin. The abnormalities in insulin resistance manifest themselves as decreased insulin-stimulated nonoxidative glucose disposal (glycogen synthesis) and increased endogenous glucose output.
glucose disposal (glycogen synthesis) and increased endogenous glucose output (Shulman, 1999). This insulin resistance is overcome by a compensatory response from the B-cells in the pancreas and euglycaemia is maintained. Eventually the hypersecretion of the B-cells causes “B-cell fatigue” and insulin levels decline with consequent appearance of hyperglycaemia resulting ultimately in frank diabetes.

In the fasting state, insulin independent tissues, the brain (~50%) and splanchnic organs (~25%) account for most of the total-body glucose disposal, insulin-dependent tissues, primarily muscle, accounts for the rest (DeFronzo et al., 1985). Hepatic glucose production (HGP) through gluconeogenesis and glycogenolysis, is mainly responsible for the glucose production during the fasting state. During prolonged fasting, levels of non-esterified fatty acids (NEFA) and ketone bodies slowly increase in the blood and help to generate the small amount of gluconeogenesis derived glucose, which is utilised by the brain. It has been estimated that after a 14h fast gluconeogenesis accounts for approximately 47% of the endogenous glucose production, and this increases to 67% after a 16 hour fast and to 97% after 42 hours (Landau et al., 1996). This percentage increase is mainly due to the reduction in glycogenolysis as the rate of gluconeogenesis does not really change when expressed in absolute values (Chen et al., 1999). Acute changes in endogenously derived NEFA show that when plasma NEFA falls so does gluconeogenesis and conversely when the levels of NEFA rises, gluconeogenesis is increased (Chen et al., 1999). These NEFA induced changes are associated with reciprocal changes in glycogenolysis. Raising plasma NEFA will partially inhibit insulin-induced suppression of endogenous glucose production i.e. cause hepatic insulin resistance mainly by interfering with insulin suppression of glycogenolysis. This supports the observation that in type 2 diabetic subjects plasma NEFA levels are often increased, which may at least in part be responsible for the relative (in relation to insulin levels) or absolute elevation in the rates of endogenous glucose production seen in these individuals.

In the postprandial state, peripheral tissue is responsible for approximately 70-90% of glucose disposal (DeFronzo et al., 1985; Shulman et al., 1990). It has been suggested that in the earliest phase of insulin resistance, the resistance occurs in the
muscle glycogen synthesis (Shulman et al., 1990). In type 2 diabetes the insulin-mediated glucose disposal is reduced by 35-40% (De Fronzo et al., 1983; DeFronzo et al., 1982; Shulman et al., 1990; Simonson et al., 1984).

1.2.2.1. Insulin-resistance

Insulin resistance, or the reduced insulin-stimulated glucose uptake, impedes glucose disposal and disrupts lipid metabolism in insulin sensitive tissues particularly muscle, the liver and adipose tissue. Most of the resistance has been attributed to a decrease in insulin-stimulated glycogen synthesis due to defects in either glycogen synthase, hexokinase or glucose transport by glucose transporter 4 (GLUT 4) (Shulman, 1999; Shulman et al., 1990). The mechanism of insulin stimulated glucose transport in human cells and adipocytes has become more clear in recent years. Briefly, the insulin molecule binds with the insulin receptor on the cell surface, this initiates a signalling cascade. The first step being the phosphorylation of the receptor and insulin-receptor-substrates such as the IRS molecules by tyrosine kinase. These substrates form complexes with docking proteins such as phosphoinositide-3 kinase (PI3K) (Sheppard and Kahn, 1999) (Fig 1.1). Subsequent steps are less clear, it is thought that PI3K induces activation of atypical protein kinase B (Akt or PKB) and protein kinase C (PKC), these in turn stimulate the insulin responsive GLUT-4 containing vesicles to translocate to the cell membrane. It is interesting to note that exercise stimulates translocation of the GLUT-4 vesicle to the cell membrane independently from the PI3K (Fig 1.1).
GLUT-4 concentrations are reduced in adipocytes but not muscle cells, of obese subjects, and subjects with impaired glucose tolerance or type 2 diabetes. As muscle is the main site for insulin stimulated disposal of glucose, reductions in GLUT-4 concentrations cannot explain the impairment of whole body insulin sensitivity. Decreased GLUT-4 production is observed with aging in muscle cells of normal subjects (Kahn, 1992), which may explain in part the observed age-related decline in insulin sensitivity.

Recent data suggest that defects in glucose transport might be responsible for the insulin resistance observed in type 2 diabetes (Cline et al., 1999). Using a novel nuclear magnetic resonance approach with carbon-13 and phosphorus-31 to measure intra-muscular glucose, glucose-6-phosphate, and glycogen concentrations under...
hyperglycaemic conditions the investigators were able to demonstrate that the rates of whole body glucose metabolism and muscle glycogen synthesis and the glucose-6-phosphate concentrations in muscle were approximately 80% lower in patients with diabetes (Cline et al., 1999). The rate-limiting step was found to be in the insulin signalling pathway that regulates translocation of intracellular GLUT-4 to the cell membrane in muscle and adipose tissue (Fig 1.1).

Several other factors have been implicated in the defects in insulin stimulated glucose transport. Early in the 1960’s Randle and colleagues introduced the concept that NEFA’s interfere with glucose utilization (Randle et al, 1963). NEFA have been shown to be chronically elevated in obesity and diabetes presumably due to increased lipolytic action (Sheppard and Kahn, 1999; Shulman, 1999). Lipid infusions in humans have been shown to inhibit IRS-1-associated phosphatidylinositol 3-kinase activity and subsequent reduction in insulin stimulated glucose transport in muscle (Dresner et al., 1999), presumably through defective translocation of GLUT-4. The defect in signalling may be caused by a NEFA induced diversion of glucose into the hexosamine pathway. Incubating isolated hepatocytes with free fatty acids of varying chain length reduced insulin binding and insulin internalisation and recycling (Svedberg et al., 1992).

NEFA levels have also been related to the diurnal variations in peripheral insulin resistance (Morgan et al., 1999). In this study NEFA levels were found to be elevated in the evening, corresponding to the increased insulin resistance commonly found at that time of the day (Morgan et al., 1999). The entero-insular axis may be affected by circulating NEFA levels. Increasing NEFA levels by heparin infusion in obese and lean subjects attenuated GIP and GLP-1 secretory responses in the obese group (Ranganath et al., 1999).

Insulin resistance is also strongly associated with increased TAG and decreased high-density lipoprotein (HDL) levels (Laakso et al., 1990). The multicentre study conducted by the European Group for the Study of Insulin Resistance studied the association between insulin resistance and lipoprotein concentration by a retrospective analysis of euglycaemic hyperinsulinaemic clamp data from 867 normoglycaemic subjects. There were significant correlations between TAG and
fasting plasma glucose and insulin levels, and mean glucose infusion rate at steady state (Fig 1.2). A positive correlation between suppression of NEFA and insulin sensitivity was also shown (Baldeweg et al., 2000).

Measurement of muscle TAG content by biopsy (Johnson et al., 1992) or intramyocellular lipid content (Perseghin et al., 1999) have shown strong relationship between increased intramuscular fat content and insulin resistance in muscle.

![Graph showing fasting plasma TAG levels plotted against quintiles of insulin sensitivity measured using the hyperinsulinaemic clamp in 867 subjects. (Baldeweg et al., 2000)](image)

“Glucose toxicity” i.e. chronic high blood glucose levels has also been implicated in the pathogenesis of insulin resistance. The mechanism proposed is that the hexosamine pathway is up-regulated, increasing levels of hexosamine metabolites that may interfere with GLUT-4 translocation and glucose transport (Sheppard and Kahn, 1999).

Paracrine factors might also play a role in the pathogenesis of insulin resistance. The cytokine, tumour necrosis factor α (TNF-α), has been found to be over-expressed in muscle and adipose tissue in obese animals and humans (Saghizadeh et al., 1996). TNF-α has shown potent inhibitory effects on tyrosine
phosphorylation of insulin receptor substrates in muscle and adipose tissue preparations (Svedberg et al., 1992). Although plasma TNF-α levels were negatively correlated with insulin stimulated glucose metabolism in humans, administration of TNF-α antibodies to humans did not improve insulin sensitivity (Ofie et al., 1996). These results were contrary to those previously found in Zucker rats (Hotamisligil and Spiegelman, 1994). Other adipocytokines which have also been implicated in the development of insulin resistance are leptin, resistin, and adiponectin. Briefly, leptin is an afferent signal molecule that interacts with the appetite and satiety centres in the brain to regulate body weight. A positive association has been found between leptin, BMI, and fasting insulin (Ahren et al., 1997). Conversely, low plasma adiponectin concentrations are found in obese individuals and in patients with diabetes and ischemic heart disease (Hotta et al., 2000). Lastly, the adipocytokine resistin has been proposed to be the link between obesity and insulin resistance as increased amounts of resistin mRNA were found in abdominal adipose depots compared to those in the thigh, suggesting an increased risk of type 2 diabetes as a result of central obesity and higher resistin levels. (McTernan et al., 2002; Steppan et al., 2001).

1.2.2.2. Insulin Sensitivity Measurements

The recognition of insulin resistance has spurred the development of measurements which are able to identify subjects at high risk of developing this syndrome. The gold standard of measuring insulin sensitivity is the euglycaemic insulin clamp technique (DeFronzo et al., 1979). However due to the difficulty of performing this test routinely, several other measurements of insulin sensitivity have also been developed. The simplest is the Homeostasis Model Assessment (HOMA) which uses only the fasting glucose and insulin values, the formula used is: HOMA= 22.5/FPG×FPI (Matthews et al., 1985). HOMA has been shown to be a reliable measure of in vivo insulin sensitivity in humans when compared with the euglycaemic insulin clamp (Bonora et al., 2000). However the Quantitative Insulin Sensitivity Check Index (QUICKI), which is based on the logarithmic and the reciprocal of the insulin-glucose product, was shown to correlate better than HOMA with insulin sensitivity across the entire spectrum of insulin sensitivity except in normal subjects (Katz et al., 2000). To correct for this, the modified Quantitative Insulin Sensitivity
Check Index (QUICKI) was developed which includes fasting NEFA levels according to the formula: QUICKI = 1/(Log (Gb) + Log (Ib) + log (NEFAb)). Where Gb, Ib and NEFAb are the fasting glucose, insulin and NEFA levels respectively. Inclusion of NEFA was shown to increase the association with insulin sensitivity in non-obese individuals (Perseghin et al., 2001). Finally the insulin sensitivity index (ISI) for whole body insulin sensitivity, developed by DeFronzo and colleagues, is calculated using OGTT plasma glucose (PG) and insulin (PI) levels, (Matsuda and DeFronzo, 1999): 10 000 divided by the square root of ([FPG x FPI] x ([mean PG x mean PI]), where PG is expressed in mg/dl (0.0551mmol/L) and PI in μU/ml (6pmol/L). All methods have been validated although, at this point of time, the QUICKI and ISI methods are probably preferred.

1.2.2.3 Insulin Secretion

It is now well established that hyperinsulinaemia precedes the development of type 2 diabetes (DeFronzo et al., 1992). A person with fasting plasma glucose levels of 6.6 mmol/L will secrete twice the amount of insulin compared to a person with a normal (3.6-6.1 mmol/L) fasting plasma glucose level. Once the fasting glucose exceeds 7.8 mmol/L, a progressive decline is seen in postprandial insulin levels (DeFronzo et al., 1989) (Fig 1.3).
Fig 1.3: Relationship of fasting blood glucose and fasting plasma insulin levels. As the fasting blood glucose levels rise above baseline there is a corresponding increase in fasting plasma insulin. Once the fasting blood glucose has reached 7.8 mol/L there is a progressive decline in fasting insulin levels. Adapted from DeFronzo et al, 1989.

Normal insulin secretion is biphasic, with an early burst of insulin release within the first 10 min, followed by a progressively increasing phase of insulin secretion that persists as long as the hyperglycaemic state is present. The early phase is important in priming the liver and other insulin target tissues. There are two stages in the development of insulin secretory defects: irregular pulsatile insulin secretion and decreased absolute insulin secretion. There is evidence that irregular pulsatile insulin secretion marks the loss of the first phase insulin secretion. It has been suggested that the loss of the first phase insulin release is the first detectable abnormality in individuals who will eventually develop diabetes (Efendic et al., 1988). The incretins such as gastric inhibitory peptide, better known as glucose-dependent insulinotrophic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) may be important determinants of the early insulin secretory response to a mixed-meal intake (Orsakov, 1992).
Almost a 100 years ago an anti-diabetogenic effect was reported using an extract from the duodenal mucous membranes (Moore et al., 1906). Already at that time the authors postulated that using an extract such as this could be used to treat diabetes. Purification of extracts which eliminated the presence of cholezystokinin-pancreozymin (CCK-PZ) demonstrated inhibitory effects on gastric acid secretion, and the purified peptide was therefore called “Gastric Inhibitory Peptide” (Brown et al., 1970). However classical experiments performed by Elrick et al. (Elrick et al., 1964; McIntyre et al., 1965) had demonstrated that intestinal peptides must also be involved in the regulation of postprandial insulin secretion. They had found that the postprandial insulin response after an oral glucose tolerance was much greater than after an intravenous glucose load which raised blood glucose levels to a level equivalent of the oral load. Subsequently, the stimulation of insulin secretion by GIP was demonstrated in humans (Dupre et al., 1973) and the alternative name: “Glucose-dependent Insulinotropic Polypeptide” was proposed which retained the original acronym.

GIP is secreted by the neuroendocrine K-cells of the duodenum and proximal jejunum (Buchan et al., 1978), the highest levels, measured by radioimmunoassay, are found in the upper jejunum (Morgan et al., 1978). Ingestion of a carbohydrate and lipid rich meal has been shown to be the main stimulus for the secretion of GIP and is proportional to the amount of glucose or fat in the meal. Secretion of GIP is therefore an indicator of the size of the meal. The biological half-life of the intact GIP (1-42 amide) is estimated to be 7 minutes, it is cleaved by the enzyme dipeptidyl-peptidase IV (DPP-IV) at the NH3 terminus to the biologically inactive fragment GIP (3-42 amide) (Meier et al., 2002).

Physiological effects of GIP include (Creutzfeldt and Nauck, 1992), increased insulin secretion in the presence of glucose (Nauck et al., 1986) and stimulation of B-cell proliferation (Trumper et al., 2001). Unique to GIP seems to be its stimulating effect on lipoprotein lipase activity and it has been shown to stimulate fatty acid
synthesis in omental adipose tissue in the rat (Oben et al., 1991). Although the secretion of GIP is normal in type 2 diabetes, the insulinotropic effect of GIP is markedly decreased (Nauck et al., 1993). It has been postulated that the lack of insulinotropic effect is due to a defect of the B-cells to respond to GIP (Meier et al., 2002). Interestingly, the response to GIP is also muted in first-degree relatives of patients with type 2 diabetes which would indicate that the impaired insulinotropic effect of GIP may be inherited and be a risk factor for diabetes (Meier et al., 2001).

1.2.2.4. Glucagon Like Peptide-1 (GLP-1)

GIP and GLP-1 share many common physiological properties, a comparison of the properties of these two incretins is summarised in table 1.1.

GLP-1 is a 30 amino acid derivative of proglucagon (PG), a 160 amino acid prohormone. PG gives rise to glucagon, GLP-1 and GLP-2 and other peptide sequences of yet unknown biological activity (Orsakov, 1992). Post-translational processing of PG occurs in the A-cells of the islets of Langerhans in the pancreas and produces glucagon, glicentin related pancreatic polypeptide, major PG fragment and a small amount of biologically inactive GLP-1. In the ileum, colon and rectum, the enteroglucagon-producing cells (L-cells) produce glicentin, oxyntomodulin, GLP-1, and GLP-2 (Fig 1.4). The amino acid sequence of GLP-1 is 100% homologous in all mammalian species (Holst, 1994; Orsakov, 1992), suggesting that is plays an important physiological role.
GLP-1 is secreted in response to intraluminal glucose (Elliott et al., 1993; Holst, 1994; Nauck et al., 1993; Orsakov, 1992). The distribution of L-cells that produce GLP-1 is greatest in the distal small intestine and colon. Nevertheless GLP-1 is released in the early phase of meal absorption (1999). The proposed mechanism for a meal induced GLP-1 stimulation is through neural signals, as it has been shown that a vagotomy blocks the GLP-1 response to intestinal lipids.

GLP-1 receptors are highly expressed on the membranes of the B-cells in the pancreas. The GLP-1 receptor belongs to the seven-transmembrane G-protein-coupled receptor family, which, in turn, belongs to the glucagon(secretin/vasoactive intestinal peptide receptor superfamily (Fehmann et al., 1995; Holst, 1994). In addition to stimulating the release of insulin, other mechanisms through which GLP-1 regulates glucose metabolism include inhibition of glucagon (Holst, 1994; Nauck et al., 1993; Nauck et al., 1997; Orsakov, 1992; Wettergren et al., 1993) and the inhibition of gastric motility (Naslund et al., 1999). The half-life of intact circulating GLP-1 is only
The two terminal NH$_2$ amino acids are cleaved from GLP-1 by the same protease which degrades GIP, DPP-IV.

The entero-insular axis is estimated to be responsible for 50% of postprandial insulin release, of this, GLP-1 and GIP together account for probably more than 80% of the intestinal incretin effect (Holst, 1994). In obese women the GLP-1 response has been found to be attenuated (Ranganath et al., 1996) but is increased in older women (Ranganath et al., 1998). Postprandial GLP-1 levels are also reduced in type 2 diabetes (Vilsbøll et al., 2001). Intravenous administration of GLP-1 will increase secretory burst mass and amplitude of pulsatile insulin secretion in both healthy individuals and those with type 2 diabetes (Ritzel et al., 2001). GIP, however tends to lose its effectiveness in patients with type 2 diabetes (Nauck et al., 1993). Therefore increasing postprandial levels of GLP-1 either through stimulating secretion, inhibition of DPP-IV, development of analogues or by injection might be a possible treatment option for type 2 diabetes.
Table 1.1: Comparison of physiologic effects of Glucose-dependent Insulinotropic Polypeptide (GIP) and Glucagon-Like Peptide-1 (GLP-1)

<table>
<thead>
<tr>
<th>Effect</th>
<th>GIP</th>
<th>GLP-1</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin secretion in normal subjects</td>
<td>Stimulation</td>
<td>Stimulation</td>
<td>(Dupre et al., 1973)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Holst, 1994; Nauck et al., 1993)</td>
</tr>
<tr>
<td>Insulin secretion in type 2 diabetes</td>
<td>Reduced stimulation</td>
<td>Stimulation</td>
<td>(Holst, 1994; Nauck et al., 1993)</td>
</tr>
<tr>
<td>Insulin extraction</td>
<td>Reduction</td>
<td>No effect</td>
<td>(Kindmark et al., 2001)</td>
</tr>
<tr>
<td>Glucagon secretion</td>
<td>No effect</td>
<td>Suppression</td>
<td>(Nauck et al., 1993; Orskov et al., 1988)</td>
</tr>
<tr>
<td>B-cell proliferation</td>
<td>Stimulation</td>
<td>Stimulation</td>
<td>(Trumper et al., 2001; Zhou et al., 1999)</td>
</tr>
<tr>
<td>Gastric emptying</td>
<td>(?)</td>
<td>Deceleration</td>
<td>(Wettergren et al., 1993)</td>
</tr>
<tr>
<td>Gastric acid secretion</td>
<td>No effect</td>
<td>Slight suppression</td>
<td>(Nauck et al., 1992; Tolessa et al., 1998)</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td>Stimulation</td>
<td>Stimulation</td>
<td>(Beck and Max, 1983; Oben et al., 1991)</td>
</tr>
<tr>
<td>Satiety</td>
<td>?</td>
<td>Enhancement</td>
<td>(Flint et al., 1998)</td>
</tr>
<tr>
<td>Body weight</td>
<td>Enhancement?</td>
<td>Reduction</td>
<td>(Zander et al., 2001)</td>
</tr>
</tbody>
</table>

Adapted from Meier et al. (Meier et al., 2002).

1.2.2.4.1 GLP-1 Analogues and DPP-IV Inhibitors

Use of GLP-1 itself as a treatment is limited due to the short half-life of GLP-1. Development of GLP-1 analogues are of therefore of great interest. Currently two peptide analogues have advanced to clinical trial: the reptilian peptide exendin-4 and a derivative of GLP-1, NN2211. Exendin-4 has a 53% sequence homology to GLP-1, is a agonist to the GLP-1 receptor and is resistant to DPP-IV (Egan et al., 2002). Single subcutaneous injections of exendin-4 decreased the fasting plasma glucose levels to the same extent as overnight administration of GLP-1 in type 2 diabetic subjects. Similar to native GLP-1, this effect was accompanied by increased levels of
insulin and decreased levels of glucagon (Kolterman et al., 2003). Administration of exendin-4 before a meal, resulted in a reduction in the rate of gastric emptying, reductions in glucagon levels with resultant reductions in postprandial glycaemia (Kolterman et al., 2003). Two longer term studies (4 weeks) with exendin-4 showed significant reductions in HbA1c (Egan et al., 2003; Fineman et al., 2003). No serious episodes of hypoglycaemia were reported in either study, however 15-30% of subjects experienced nausea.

The second analogue used in clinical studies is a long-acting derivative of GLP-1: NN2211. This compound is composed of GLP-1 covalently linked to a hexadecyl residue, this causes it to bind to albumin which extends the half life of the GLP-1 NN2211 to approximately 12h (Elbrond et al., 2002). Administration of a single subcutaneous injection increased meal-stimulated insulin levels and lowered postprandial glucose levels (Juhl et al., 2002). As with exendin-4, gastrointestinal side effects again occurred. Long-term trials will need to be undertaken to assess the clinical utility of these products.

To overcome the limitation of the extremely short half-life of GLP-1, DPP-IV inhibitors have been developed. The DPP-IV inhibitor NVP-DPP728 has been reported to reduce glucose and insulin levels throughout the day, and reduce HbA1c and fasting plasma glucose levels after 4wks of treatment in type 2 diabetic subjects (Ahren et al., 2002).

Whichever mechanism is used, elevation of GLP-1 levels might therefore be a valuable treatment strategy in type 2 diabetes.

1.2.3. Treatment of Type 2 Diabetes

Treatment of diabetes is now more aggressive as tight glucose control has been shown to reduce the risk of microvascular complications in both type 1 and 2 diabetes (Diabetes Control Trial, 1996; Turner et al., 1996). Diet remains the cornerstone for the treatment of diabetes. Dietary guidelines vary to some extent between countries and agencies, a comparison of recommendations as given by the European
Association for the Study of Diabetes (EASD), the Canadian Diabetes Association and the American Diabetes Association are given in table 1.2.

Table 1.2: Comparison of international nutritional guidelines for the treatment of type 2 diabetes.

<table>
<thead>
<tr>
<th>Agency</th>
<th>EASD</th>
<th>CDA</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of Publication</td>
<td>2000</td>
<td>2000</td>
<td>2002</td>
</tr>
<tr>
<td>Protein</td>
<td>10-20%</td>
<td>15%</td>
<td>15-20%</td>
</tr>
<tr>
<td>Fat:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25-30%</td>
<td>25-30%</td>
<td></td>
</tr>
<tr>
<td>Saturated</td>
<td>&lt;10% (incl. trans)</td>
<td>≤10%</td>
<td>&lt;7-10%</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>60-70% (incl. CHO)</td>
<td>60-70% (incl. CHO)</td>
<td>~10%</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>&lt;10%</td>
<td>≤10%</td>
<td></td>
</tr>
<tr>
<td>Transunsaturated</td>
<td>Low intake</td>
<td>Low intake</td>
<td>Low intake</td>
</tr>
<tr>
<td>Carbohydrate:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibre</td>
<td>High Fibre</td>
<td>25-35g</td>
<td>-</td>
</tr>
<tr>
<td>Type of CHO</td>
<td>Low GI</td>
<td>Low GI</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>&lt;300mg</td>
<td>-</td>
<td>&lt;200-300mg</td>
</tr>
<tr>
<td>Reference</td>
<td>[EASD, 2000]</td>
<td>(Wolever et al., 1999)</td>
<td>[ADA, 2002]</td>
</tr>
</tbody>
</table>

EASD, European Association for the Study of Diabetes; CDA, Canadian Diabetes Association; ADA, American Association Diabetes Association.
As can be seen from table 1.2, recommendations from the EASD and CDA are very similar, the notable exception is the ADA, who did not support the concept of the glycaemic index (GI) or a high fibre diet. Their position was that, although low glycaemic index foods may produce a lower postprandial glycaemic response, there is not enough long-term evidence in either type 1 or type 2 diabetes to warrant use of the glycaemic index as a treatment strategy. In addition they suggested that the likelihood of people adhering to such a diet was low [ADA, 2002]. However a recent statement issued by the ADA (Sheard et al., 2004) reversed this position and the ADA now allows that the glycaemic index “can provide an additional benefit over that observed when total carbohydrate is considered alone”. A meta-analysis of the effect of low glycaemic index diets by Brand-Miller et al (Brand-Miller et al., 2003) showed that low GI diets had a small but clinically significant effect on medium-term metabolic control of diabetes which was comparable to pharmaceutical agents which also lower postprandial glycaemia. Currently a multi-centre trial on the effectiveness of the GI is underway in Toronto, which hopefully will help establish the usefulness of the GI in the treatment of diabetes in the near future.

Many medications are available for the treatment of diabetes. A brief description of the different available drugs is given together with their mechanism of action:

**Sulphonylureas**, such as glyburide, glipizide and glimepiride which stimulate the secretion of insulin by augmentation of potassium channel activity in the pancreatic islet cells.

**Meglitinides**, non sulphonylurea agents that are also insulin secretagogues;

**Biguanides**, such as metformin, reduces hepatic glucose production in some patients and increases peripheral utilization;

**Alpha-glucosidase** inhibitors such as acarbose, competitively inhibits the ability of enzymes in the small intestinal brush border to break down oligosaccharides and disaccharides into monosaccharides. In this way these drugs effectively compensate for the defective early-phase insulin release by slowing the absorption of sugars from carbohydrate;

**Thiazolidinediones** such as Rosiglitazone and Pioglitazone appear to activate peroxisome proliferator-activated receptor gamma, leading to increased glucose
transporter expression. Improvements in insulin sensitivity have been observed in liver, muscle and adipose tissue.

Finally, there is an extensive choice of short, intermediate and long acting insulins.

Although all of the above mentioned drugs are of undoubted benefit there are also drawbacks: sulphonylureas can induce weight gain and hypoglycaemia, alpha-glucosidase inhibitors often are accompanied by gastric symptoms, and adverse reactions to metformin are not uncommon. In addition, despite the range of medications available, diabetes control often remains sub-optimal, therefore new treatments continue to be of interest.

Two new novel treatments we have explored are the viscous fibre, konjac-mannan and the root, American ginseng. A description of the properties and studies utilising these two compounds follows:

1.3. Viscous Fibres in General and the Viscous Fibre Konjac-mannan (Amorphallus Konjac, K Koch) in Particular

1.3.1 Introduction

Epidemiological studies have suggested a strong link between intake of dietary fibre and reduced risk of heart disease (Bazzano et al., 2003). A recent review identified 9 prospective trials which examined the relationship between risk of developing coronary heart disease (CHD) and dietary fibre intake (Kushi et al., 1999). Seven out of the 9 trials found a significantly lower risk of CHD in those who consumed greater amounts of fibre. Results from studies which specifically looked at the effect of soluble fibre, are less consistent (Bazzano et al., 2003; Kushi et al., 1999; Liu et al., 2002; Pietinen et al., 1996). Only two of these studies showed a significant, inverse relationship between soluble fibre and risk of CHD after adjustments were made for multiple confounding risk factors (Bazzano et al., 2003; Pietinen et al.,
1996). These disappointing results might have been due to the many confounding risk factors in epidemiological trials, or alternatively it might be due to the relatively small amount of soluble fibre found in a typical, low fibre, “Western” diet, which might be insufficient to exert a significant beneficial effect.

Soluble fibre is of interest however as it has been shown to improve postprandial glycaemia in acute studies and reduce cardiovascular risk factors such as serum cholesterol levels and especially LDL-cholesterol levels in longer-term randomised controlled trials.

1.3.2. Mechanism of Action of Viscous Fibres

Early work demonstrated that incorporation of viscous, un-absorbable plant polysaccharides such as guar and pectin into test meals, reduced postprandial glycaemia in healthy and type 2 diabetes subjects (Jenkins et al., 1976; Jenkins et al., 1977). The effect was independent of endogenous insulin secretory ability (Jenkins et al., 1976). It had been observed that fibres with the greatest viscosity caused the greatest reduction in postprandial glycaemia (Jenkins et al., 1978). It was hypothesised that the reduced postprandial glycaemia observed was due to the ability of the fibre to form a gel in the small intestine which slowed the rate of diffusion of nutrients from the small intestine (Jenkins et al., 1978). This hypothesis was confirmed by a study in pigs which had been fed either a control diet or a diet to which guar gum was added at a concentration of either 20 or 40g/kg. Blood samples were taken simultaneously from the hepatic portal vein and the mesenteric artery. Jejunal digesta was also collected. Absorption of glucose was significantly reduced by both guar concentrations and was directly related to the concentration of the guar in the diet. Furthermore there was an inverse relation between the viscosity of the jejunal digesta and the rate of glucose absorption (Ellis et al., 1995). Reduction in gastric emptying might also play a part in the mechanism of action of viscous fibres (Leeds et al., 1981) but not malabsorption (Jenkins et al., 1978).

Guar administration is also associated with a sustained suppression of 3-hydroxybutyrate and NEFA production (Jenkins et al., 1980). In addition,
postprandial levels of GIP (Ellis et al., 1995; Jenkins et al., 1980; Morgan et al., 1979) and enteroglucagon (Jenkins et al., 1980) are reduced when guar is added to a test meal, demonstrating that the gut endocrine response can be manipulated by interventions which alter the pattern of carbohydrate absorption.

Several factors have been implicated in the mechanism of action for the lipid lowering effect seen with viscous fibre: increase in faecal neutral sterol and bile acid excretion (Chen et al., 2003), inhibition of cholesterol absorption in the jejunum (Ebihara and Schneeman, 1989), reduced bile acid absorption in the ileum (Kiriyama et al., 1974) and improvement in peripheral insulin sensitivity (Vuksan et al., 1999a).

As viscosity has been shown to be important in the efficacy of viscous fibres, konjac mannan, a novel source of fibre, is of interest in that it represents a polysaccharide with one of the highest viscosities (Doi et al., 1981) (Fig 1.5).

Fig 1.5. Changes in viscosity with time of pectin, guar gum and konjac flour (Doi et al., 1981).
1.3.3. **History and Chemical Structure of Konjac Mannan Fibre**

The tuber root of the konjac plant has been used for over a thousand years in Asia as a foodstuff and a folk remedy. Written evidence of the use of konjac as a food in China and Japan appears in the sixth century AD in an ancient Japanese work entitled, “Man-you-shuu”. Historically, konjac was used to “cleanse one’s digestive tract of irritating and poisonous substances and keep one’s internal organs clean” [Konnyaku Inc, 2004].

Konjac, a perennial plant, is unique to Asia and specially cultivated in Japan (Fig 1.6). By grinding the root, flour is obtained which is then made into a rubbery gel. Konjac flour also has been used in the United States since the turn of the century and is listed by the FDA on the “Generally Recognised as Safe” list and in Canada it has “novel food” status.

The flour's main constituent is a highly viscous mannan, a polysaccharide chain containing glucose and mannose in a molar ratio of 1:1.6 with B-1-4 linkages (Fig 1.7).

![Fig 1.6: Drawing of a konjac plant showing the root from which the viscous fibre is extracted](image)

![Fig 1.7: Structure of konjac mannan fibre.](image)
1.3.4 Acute Studies with Konjac Mannan Fibre

When 20g of glucose was fed to healthy subjects alone or with 3 grams of fibre added from either psyllium, xanthan or konjac, the results matched the relative viscosity of the fibres. In other words, the fibre with the lowest viscosity (psyllium) had the highest postprandial glucose results, and konjac, which has the highest viscosity, gave the lowest postprandial glucose results. The response after xanthan was intermediate between psyllium and konjac (Fig 1.8)(Kim et al., 1996). This study again confirmed the viscosity hypothesis.

Fig 1.8: Postprandial glycaemic responses to a 20 g oral glucose challenge done alone (O) or following 3 g psyllium (◇), xanthan (□), or konjac mannan (▲). Time points at the same time interval with different letters are significantly different (p<0.05). Adapted from (Kim et al., 1996)

Combining konjac mannan with another polysaccharide can increase the viscosity significantly (Proprietary Technology: Provisional U.S Patent #60/208,090), this increase in viscosity is the result of the interaction of the cellulosic backbone of other polysaccharides and the mannan backbone of konjac (Kim et al., 1996; Tye, 1991). The large quantity of fibre needed to cause a significant metabolic effect has always been one of the drawbacks of viscous fibre; the advantage of a highly viscous fibre blend would be that the dose could be significantly reduced and therefore enhance palatability. Our group studied the effect on glycaemic response of incorporating a high viscosity konjac mannan polysaccharide blend into a biscuit in
both healthy subjects and subjects with type 2 diabetes (Figs 1.9 and 1.10 respectively) (Vuksan et al., 1992). Three grams of the konjac fibre blend was added to 50 grams of available carbohydrate from the biscuit and compared to standard white bread also containing 50g carbohydrate. The konjac fibre blend significantly reduced the postprandial glycaemia both in healthy subjects and in subjects with type 2 diabetes. The glycaemic index of the fibre containing biscuits was 26±5 and 37±6 in healthy and in type 2 diabetes subjects respectively (Vuksan et al., 1992). These very low glycaemic index values were achieved using almost pharmacological doses of konjac mannan and so improving the therapeutic potential of this fibre.

Fig 1.9: Incremental Changes in Glycaemia and Area under the Blood Glucose Curve (as glycaemic index) between ProMannan® (konjac fibre blend) Biscuits and White Bread in 9 healthy subjects. Results are expressed as Mean±SEM, *p<0.05, **p<0.001. Adapted from (Vuksan et al., 1992)
1.3.5 Longer-term Studies with Konjac mannan fibre

Like other viscous fibres, konjac mannan has been shown to lower total-cholesterol, LDL-cholesterol and triacylglycerol (Arvill and Bodin, 1995; Chen et al., 2003; Venter et al., 1987), systolic blood pressure (Arvill and Bodin, 1995) and glycaemia (Doi et al., 1979) when taken as a supplement. Earlier, two trials were conducted in our laboratory, one in type 2 diabetic subjects and the second in individuals with insulin resistance syndrome (Vuksan et al., 1999b; Vuksan et al., 2000b). In the first study, konjac mannan was incorporated into biscuits and given to patients with type 2 diabetes over a three-week period. The patients also had medically treated hypertension and hyperlipidaemia. Serum fructosamine, serum cholesterol, LDL/HDL ratio, apolipoprotein B/A ratio, and systolic blood pressure were all significantly reduced following the konjac mannan supplemented diet (Vuksan et al., 1999b). In the second study, a 3 week cross over, placebo controlled metabolic trial, individuals with insulin resistance syndrome were treated with konjac (Vuksan et al., 2000b). Again, significant reductions in serum fructosamine, total cholesterol:HDL-cholesterol ratio, and apolipoprotein B were observed (Fig 1.11). In addition, an improvement in insulin sensitivity using the ISI method (Matsuda and DeFronzo, 1999), was observed after 3 weeks on the fibre blend.
Fig 1.11: Percent change in serum cholesterol (Total-C), LDL-cholesterol, Total:HDL-cholesterol, LDL:HDL-cholesterol and Apolipoprotein A-1:B after 3 weeks of supplementation with either a konjac fibre blend (Promannan®) or wheat bran (control) biscuits in 11 subjects with impaired glucose tolerance. (Vuksan et al., 1992)

Compared to four other soluble fibres (guar, pectin, psyllium and oat) the lipid lowering ability of konjac was shown to be 2-4 times greater (Brown et al., 1999). The degree to which the konjac fibre blend improved long-term glycaemic control was comparable to trials using Acarbose.

1.3.6 Viscous fibre and the Second Meal Effect

Viscous fibre, when taken with breakfast, has been shown to influence the subsequent meal. When healthy subjects consumed two 80 g oral glucose loads, 4h apart. Addition of 22.3g of guar to the first load decreased the rise in blood glucose and insulin after the fibre free second meal by 50% and 31% respectively (Jenkins et al., 1980). Both 3-hydroxybutyrate and NEFA tended to rise before the second meal when the breakfast contained no fibre. No changes in GIP and enteroglucagon were observed (Jenkins et al., 1980).

Viscous fibre might therefore have a potential therapeutic role in the treatment of type 2 diabetes providing a suitable and effective mode of administration is available.
1.4. **American Ginseng** (*Panax quinquefolius* L.)

An increasing number of people are turning to alternative therapies such as diet supplements and herbs. In the case of herbal remedies, their consumption has increased by approximately 380% from 1990-1997 in the US with annual sales exceeding $300 million (Eisenberg et al., 1998). This increase has been observed despite lack of evidence to support the therapeutic value of many of the herbal remedies. As one of the most popular herbal remedies is ginseng (Eisenberg et al., 1998), trials establishing the medical efficacy and safety of ginseng are urgently needed.

Earlier studies which have been carried out on ginseng often do not specify type, age, what part of the root has been used, preparation, ginsenoside profile etc (Vogler et al., 1999). There are thirteen distinct species of ginseng, the most commonly used species are: American ginseng (*Panax quinquefolius* L.), Asian (*Panax ginseng* C.A. Meyer), Japanese (*Panax Japonicus* C.A. Meyer), san-chi (*Panax Notoginseng* Burke F.H. Chen), and the non-panax species Siberian (*Eleutherococcus Senticosus*). American ginseng is indigenous to Ontario, Quebec, British Colombia and Wisconsin, while the other species are indigenous to Asia. Many beneficial effects are ascribed to ginseng, it is often described as an “adaptogen” in that it is supposed to “increase the resistance against noxious or stressful influences without impairing physiological functions” (Chong and Oberholzer, 1988). In traditional Chinese medicine it is used as a tonic and to generally enhance well being. At the present time claims for ginseng include: improvement of immune function, exercise endurance, sexual function, glucose metabolism and reduced risk of cancer and cardiovascular disease (Vogler et al., 1999). Evidence is emerging suggesting a role for ginseng in the treatment of diabetes.
1.4.1. Mechanism of Action of Ginseng on Glucose Metabolism

The mechanism of action of ginseng has been largely unexplored. However, based on animal studies, several mechanisms of action of ginseng have been suggested: 1. increase in insulin secretion, 2. changes in glucose transport and disposal and 3. slowing of gastric emptying (Hashimoto et al., 2003).

1.4.1.1. Increase in insulin secretion

We observed that administration of 6g of American ginseng increased the insulin secretion twofold in the first 45min after a 75g oral glucose challenge (Vuksan et al., 2000a). Although it was not possible to measure the first phase insulin response i.e. the response over the first 5-10min, the relatively early increase of insulin suggests that ginseng might be able to influence, if not the first phase, but certainly the early phase of insulin secretion. Animal studies support these observations. DPG-3-2, a component of ginseng radix, the root of Panax ginseng, lowers the blood glucose level and stimulates insulin release in diabetic animals, and was shown to stimulate insulin biosynthesis in islets from KK-CAy mice (Waki et al., 1982). Perfusion of isolated rat pancreas with a ginseng extract from ginseng radix, stimulated an increase in insulin release that was similar to the increase seen when sulphonylurea was used. Using the same extract in alloxan diabetic rats, the insulin response was increased after ginseng to or above the control responses in normal rats (Kimura et al., 1981).

1.4.1.2. Changes in glucose transport and disposal

A water extract of Panax ginseng administered to normal and epinephrine-induced hyperglycaemic mice increased GLUT-2 protein in the liver (Ohnishi et al., 1996). In sheep erythrocytes, Panax ginseng extract increased rates of glucose uptake and transport in a dose-dependent manner (Hasegawa et al., 1994).

In rat skeletal muscle and adipose tissue, ginseng has been shown to mediate the insulin stimulated glucose uptake (Roy et al., 1998). The ginsenoside Rb2, increased the activity of the rate limiting glycolytic enzymes, glucokinase and phosphofructokinase, while decreasing the activity of the rate limiting gluconeogenic
enzyme glucose-6-phosphatase in rat liver preparations (Hasegawa et al., 1994; Kimura et al., 1981; Yokozawa et al., 1985a).

1.4.1.3. Slowing of gastric emptying and absorption

Panax ginseng has been shown to reduce carbachol-induced accelerated small intestine transit time in mice, the authors suggested that both an inhibitory effect on cholinergic nervous system and direct suppressive effect on muscles were involved in the attenuation of the hyper peristalsis (Satoh et al., 2001). Another study demonstrated a reduction of glucose and maltose absorption in human duodenal mucosa but at the same time they also reported an increase in duodenal muscle movement (Onomura et al., 1999). However these studies all used Chinese ginseng and it is not clear what the effect of American ginseng is, if any, on gastric motility and absorption.

The ginseng root contains many potentially active ingredients such as ginsenosides, polysaccharides, flavanoids, daucosterin, mucilaginous substances, amino acids, vitamins etc. (Wang et al., 1999; Zhang et al., 1979; Zhang et al., 1980). The active ingredients of ginseng are generally assumed to be the ginsenosides (Chen, 1983; Hasegawa et al., 1994; Ng and Yeung, 1985).

1.4.2. Ginsenosides

1.4.2.1. Structure and Concentration

The accepted nomenclature of the individual saponins named Rx (x = a, a,b1,c,d,e,f,g1,g2...) is based on the sequence of spots detected after silica gel thin layer chromatography. Ginsenosides are tri-terpenoid glycosides (saponins) of the dammaran series. They can be divided into three types: oleanic acid, panaxadiol and panaxatriol types. Twenty-eight ginsenosides have been identified in the root, stems, leaves and flowers and flower-buds of the ginseng plant (Cai P.L., 1982; Kuang and Xu, 1982; Shao and Xu, 1982; Zhang et al., 1979; Zhang et al., 1980)(Fig 1.13).
Fig 1.13: Structure and names of the 3 main ginsenoside classes and examples of the most common ginsenosides isolated from Panax Ginseng.

The amount and proportion of ginsenosides changes with the number of years the ginsengs root is left to grow (Zhang et al., 1980) (Table 1.3), with the highest concentrations occurring generally at 4-5 years.
Table 1.3: Comparison of Saponin content of ginseng roots, which have been grown for 2-9 years (Zhang et al., 1980).

<table>
<thead>
<tr>
<th>Years</th>
<th>Total Saponins (%)</th>
<th>Rb (%)</th>
<th>Rg (%)</th>
<th>Ro (%)</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>1.97</td>
<td>0.88</td>
<td>0.54</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>2.2</td>
<td>1.03</td>
<td>0.62</td>
<td>0.17</td>
</tr>
<tr>
<td>4</td>
<td>4.75</td>
<td>2.27</td>
<td>1.1</td>
<td>0.40</td>
</tr>
<tr>
<td>5</td>
<td>4.6</td>
<td>2.08</td>
<td>1.19</td>
<td>0.21</td>
</tr>
<tr>
<td>6</td>
<td>3.84</td>
<td>1.94</td>
<td>0.81</td>
<td>0.29</td>
</tr>
<tr>
<td>9</td>
<td>3.81</td>
<td>2.32</td>
<td>0.46</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Ginsenoside content and profile varies between species and within species. For example American has a higher ginsenoside content than Asian while Siberian ginseng has none (Attele et al., 1999). Presence or absence of certain ginsenosides can be used to identify the species, e.g. the ginsenoside Rf is found in Asian ginseng but not in American ginseng. (Li et al., 2000). Similarly, ratios of ginsenosides can also be used to identify species, a ratio smaller than one in the ginsenosides Rg1/Re and Rb2/Rc distinguishes American from Asian ginseng (Chan et al., 2000). The variability of ginsenoside content and amount between species emphasises the importance of specifying the type of ginseng used, it has been estimated that the ginsenoside concentration varies as much as 15-fold in capsules (Harkey et al., 2001).

As mentioned earlier, ginsenoside content varies with the age of the root but also with the location where it has been grown. Ginsenosides are not only found in the root of the plant, but also in the leaves, stem, berries, root and rootlets. The ginsenoside content of American leaves has been shown to be 1.4 fold higher than in the root of the same plant. Even though traditional Chinese medicine considers products made from the small rootlets to be inferior, the ginsenoside content has been estimated to be double than in the prized root body (Liu and Xiao, 1992).
1.4.2.2. Ginsenoside Absorption and Metabolism

A recent study showed that intact ginsenosides in addition to hydrolysates can be absorbed from the human digestive tract and be measured in the circulation and in the urine (Tawab et al., 2003). Absorption of ginsenosides is estimated to be quite low (less than 5%) (Tawab et al., 2003). It has been estimated that the ginsenosides from American ginseng have a half life of about 8 hours once they reach the systemic circulation in the rabbit (Chen and Staba, 1980).

1.4.2.3. Physiological Role of Individual Ginsenosides

Using individual ginsenosides, hypoglycaemic effects have also been observed. Administration of the propanaxatriol Rg1 at 50mg/kg by stomach intubation, decreased glycaemia by 17% compared to placebo in resting rats who had been fasting for over 24h (Martinez and Staba, 1984). The 20(S)-protopanaxadiol ginsenoside Rb1, has been shown to increase glucose uptake into sheep erythrocytes in a dose dependent manner (Hasegawa et al., 1994). The protopanaxadiol Rb2 reduced glycaemia in streptozotocin induced diabetic rats after 6 days (Yokozawa et al., 1985c) and has been shown to increase the activity of the rate limiting glycolytic enzymes glucokinase and phosphofructokinase, while decreasing the activity of the rate limiting gluconeogenic enzyme glucose-6-phosphatase in rat liver preparations (Ng and Yeung, 1985). However, hyperglycaemic effects have also been observed. Insulin stimulated 2-deoxy glucose uptake was significantly inhibited in a dose dependent manner by a water extract of Asian ginseng when compared to control in 3T3-L1 adipocytes (Hong et al., 2000).

Considering the intra and inter-species variability of ginsenoside content these different effects might to some extent explain the contrary reports in the literature.

1.4.3. Acute Studies with Ginseng

Animal studies have shown significant hypoglycaemic action by a variety of ginsengs (e.g. American, Chinese, Siberian and Korean) (Martinez and Staba, 1984; Oshima et al., 1987) using either the root or an extract of the root. Dosages used in
animal experiments are often much larger than used in human experiments and are therefore difficult to interpret. However several acute human studies with ginseng have been undertaken, including previous studies by our group. In a series of 4 studies, our group reported that American ginseng reduced postprandial glycaemia by 15-45%, when administered either 30-40min before, or together, with a 25g oral glucose challenge in subjects with diabetes, and only when given 30-40min before the challenge in normal subjects (Vuksan et al., 2001a). To identify the optimum dose needed, doses ranging from 1-9g were used, but all were equally efficacious (Vuksan et al., 2000a; Vuksan et al., 2000c) suggesting that the maximum effect has already been reached with the 1g dose.

Unlike viscous fibre, which reduces both postprandial glycaemia and insulinacmia, ginseng decreases postprandial glycaemia but causes an increase in postprandial insulinaemia (Fig 1.14). This confirms one of the postulated mechanisms of action for ginseng (see 1.4.1.1.). In addition, since the effect of ginseng was seen to be the same whether the ginseng was administered together or 40, 80 120 min prior to the meal in type 2 diabetic subjects, they concluded that ginseng might therefore act by increasing the glucose-dependent insulin response (Vuksan et al., 2000c).
The ability of other ginsengs types to influence postprandial glycaemia and insulinaemia was also investigated. Eight different ginseng types were studied: American, wild-American, Asian, Korean red, Siberian, Japanese, Sanchi and Vietnamese ginseng (Sievenpiper et al., 2002). There was a significant effect of ginseng type on 90min and AUC glycaemia and AUC insulinaemia \( (p<0.05) \). However the individual results were variable, American ginseng again lowered postprandial glycaemia but Asian ginseng and the non-ginseng species, Siberian increased postprandial glycaemia. To determine the role of ginsenosides a stepwise multiple regression was performed which showed that an increase in protopanaxadiol to protopanaxatriol \((\text{PPD:PPT})\) ginsenoside ratio was an independent predictor for reductions at 90min in glycaemia \((\beta =1.39, r^2=0.07)\) and insulinaemia \((\beta =0.26, r^2=0.07)\) and in glucose AUC \((\beta =0.25, r^2=0.06)\). Nevertheless they concluded that, based on the small \(r^2\) values, other components are also involved (Sievenpiper et al., 2002).

### 1.4.4 Long-term Trials with Ginseng

Limited long-term data are available. Of the three long-term human studies conducted with ginseng, the first showed that supplementation for 8 weeks with 100mg or 200mg/daily of a non-specified type of ginseng extract resulted in reductions of HbA1c (Sotaniemi et al, 1995). Results of this study are difficult to interpret because of weight loss in the subjects taking ginseng. In the second study, 24 months of treatment of 3-4.5g/day with Korean ginseng in subjects with diabetes resulted in reductions in HbA1c, however primary sources for this article could not be found and no further details were reported (Tetsutani et al., 2000). The third long-term study was conducted by our group. Using an American ginseng extract in an 8-week double-blind placebo controlled crossover trial in 24 subjects with diabetes, reductions in fasting glucose, HbA1c, blood pressure and lipids were observed. Dosage used was 1g ginseng extract taken 40 min before each meal to provide a total of 3g/day. A non-significant increase of 25% in fasting insulin levels was also observed (Vuksan et al., 2001b; Vuksan et al., 2001c) (Fig 1.14).
Fig 1.14. Comparison of percent change from baseline in fasting plasma glucose, insulin, and HbA1C between placebo and American ginseng following 8 weeks of supplementation in 24 type 2 diabetic subjects. P values are for the between treatment difference for absolute values (ANCOVA GLM). Data are mean±SEM. FPG=fasting plasma glucose, FPI=fasting plasma insulin. (Vuksan et al., 2001b)

1.4.5. Ginseng and Adverse Effects

Based on anecdotal evidence, concerns have been raised that ginseng may increase blood pressure (Siegel, 1980). Apart from increases in blood pressure this study also reported that 14 out of 133 patients who used ginseng regularly experienced diarrhoea, nervousness, insomnia, and skin eruptions. However the methods used in this report have been criticised. Recently, Han et al (1998) evaluated the changes of diurnal blood pressure in essential hypertensive patients prior to and after 8-weeks of 4.5g/day Korean red ginseng treatment. The study demonstrated that 24hr mean systolic blood pressure (SBP) decreased significantly, with the largest decreases observed at daytime (8am-6pm) and dawn (5am-7am). Animal data also support these results. Kim et al (1999) demonstrated that increasing levels of a ginsenoside extract relaxed contractions of aortic rings in the presence, but not in the absence of the endothelium. These vascular relaxations were mediated by the release of NO, which enhanced accumulation of cyclic guanosine monophosphate (cGMP) in the aortic rings. Rhodcs and collcagues (1995) showed that ginseng enhanced formation of citrulline from added arginine, implying synthesis of NO. In our long-term study, 8 weeks of 3g/day of American ginseng reduced SBP by 5.6mmHg (p=0.03) and increased nitric oxide (NO) generation by 6.7% (p=0.03) (Xu Z, 2000).
Mania has also been related to ginseng intake (Engelberg et al., 2001). However most of these side effects have been associated with Asian ginseng and no adverse effects have been reported with American ginseng (McGuffin, 1997). The WHO continues to endorse ginseng as a herb without known side-effects (Health Canada, 1999).

1.4.6 Ginseng - Summary

Both acute and long-term studies with American ginseng indicate a possible therapeutic role of ginseng in the treatment of type 2 diabetes. Although the exact hypoglycaemic mechanism of American ginseng is still elusive, studies from our laboratory strongly suggest enhancement of insulin secretion.

1.5. Rationale for Combining Ginseng and Konjac

Our studies indicate that both ginseng and konjac mannan improve diabetes control and reduce cardiovascular risk factors such as serum cholesterol and hypertension. The most effective treatment of type 2 diabetes includes increasing insulin sensitivity and/or B-cell secretion. Potentially ginseng and konjac can do both: Ginseng might act through increasing insulin secretion during the early part of the meal (Vuksan et al., 2000a). Its action therefore could be compared to sulphonylurea, which also has an insulinotropic effect on pancreatic B-cells. The difference between ginseng and sulphonylurea however is that ginseng only seems to stimulate insulin release in response to a meal (i.e. a glucose-stimulated insulin secretion). This has been illustrated by the acute studies which administered American ginseng 40 minutes before the meal and showed that neither glucose or insulin levels changed over the 40 min period (Vuksan et al., 2000a). The advantage for patients taking ginseng would be a reduced risk of hypoglycaemic episodes in the case of postponed or missed meals in comparison to patients on sulphonylurea treatment.

Konjac acts through slowing carbohydrate absorption, and so decreasing the postprandial glycaemia. Thus the effect of konjac can be compared to other means of prolonging carbohydrate absorption including other viscous fibres, low glycaemic...
index starchy foods, increased food frequency and α-glucosidase inhibitors (e.g. Acarbose) used therapeutically in the treatment of diabetes. In addition konjac might also improve insulin sensitivity, the mechanism of action of metformin.

Combining ginseng and konjac might therefore result in an additive or synergistic effect on postprandial metabolic parameters by meeting two major physiological requirements in the treatment of type 2 diabetes.

1.6 Aims and Objectives of Current Research

The aims of this project were first to establish the acute effect of using the combination of konjac mannan fibre and American ginseng on postprandial glucose metabolism in type 2 diabetes. At the same time the effect of konjac and ginseng on GI hormones, TAG and NEFA will be determined as these might provide additional information on the mechanism of action of these two compounds. Guar gum has been shown to reduce postprandial glycaemia to a second or subsequent meal, but it is not known whether konjac mannan exerts the same effect or whether ginseng affects the response to a second meal. The acute study will therefore be extended to include a lunch, or “second meal”, which will allow determination of the effect, if any, of a breakfast containing konjac and/or ginseng on the metabolic response to this 2nd meal.

If the combination of konjac and ginseng is shown to be effective in modulating postprandial glycaemia and insulinaemia, a randomised, crossover long-term study in subjects with type 2 diabetes will be undertaken. Both treatment arms of the study (konjac+ginseng vs control) will be of three months duration. The main outcome of the long-term study will be HbA1c, but other parameters such as risk factors for cardiovascular disease will also be measured. It is anticipated that prior to the long-term study, preliminary work will need to be undertaken to establish the optimum administration mode of the konjac fibre and effectiveness of any the supplements formulated.
The objectives of the following series of experiments were therefore as follows:

1. To establish the acute effects of konjac mannan and ginseng alone and in combination on postprandial glycaemia in subjects with type 2 diabetes.

2. To establish the acute effects of konjac mannan and ginseng on insulin and GI hormone secretion in subject with type 2 diabetes.

3. To establish the effect konjac and/or ginseng on the postprandial glucose and insulin response to a second meal.

4. To formulate effective and palatable products containing konjac mannan.

5. To establish the combined effect of konjac mannan and ginseng on metabolic control during a three months, randomised crossover trial in subjects with type 2 diabetes.
Chapter 2

MATERIALS AND METHODS

This chapter describes the materials and methods used in the experimental work. Protocols for individual experiments will be described in the relevant chapters.

2.1 Equipment and Materials

2.1.1. Equipment

<table>
<thead>
<tr>
<th>Company</th>
<th>Equipment</th>
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</thead>
<tbody>
<tr>
<td>D.W. Brookfield Ltd, Cooksville, ON</td>
<td>Synchro-electric Viscometer</td>
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<tr>
<td>Fisher Scientific, Canada</td>
<td>Thermix Stirrer Model 1205</td>
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<tr>
<td>Becton Dickinson, Franklin Lakes, NJ</td>
<td>Vacutainers:</td>
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<td></td>
<td>Sodium Fluoride Potassium Oxalate (2,5&amp;7ml)</td>
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<td></td>
<td>Potassium EDTA (5ml)</td>
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<td></td>
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<tr>
<td></td>
<td>SSY Gel and clot activator (6ml)</td>
</tr>
<tr>
<td></td>
<td>No additives (10ml)</td>
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<tr>
<td>Medex, Ohio</td>
<td>Injector adapter (catheter)</td>
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<td>Futrex 5000, total body fat measurements</td>
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<tr>
<td>Sarstedt Inc, St Leonard, Quebec</td>
<td>Plastic tubes with caps, 7mL</td>
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<td>Material</td>
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<tr>
<td>----------------------------------------------</td>
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</tr>
<tr>
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<td>Omron Healthcare Inc</td>
<td>Omron HEM-907 digital blood pressure device</td>
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2.1.2. Materials:

<table>
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<tr>
<td>American Association of Cereal Chemists, St Paul, MN, USA</td>
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<td>International Nutrition Ltd, Markham, Ontario</td>
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<tr>
<td>Chai-Na-Ta Corporation, Langley, British Colombia</td>
<td>Ginseng capsules</td>
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<tr>
<td>Ensure Abbott Laboratories Limited, Saint-Laurent, Quebec, Canada</td>
<td>Ensure liquid meal replacement</td>
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Rougier Inc, Chambly, Quebec | Glucodex, 75g glucose drink
SIGMA Chemical Company, St Louis, USA | Aprotinin
FiberJohnson, Toronto, On, Canada | konjac mannan KJM 3, mesh size unknown
Fiber Tech Co, Gyeonggi, Korea | Konjac mannan fibres: KJM1: 100-250 mesh size, KJM 2: 40-80 mesh size
Gumix International Inc, Fort Lee, NJ, USA | Xanthan gum Type KF, control no 705/43/18907
Grain Process Enterprises Ltd, Scarborough, Ontario | White wheat bran control supplement for long term study
Broda Farm, ON | Ginseng root
Kensington Bakery, Toronto, ON | Viscous fibre blend bread
Robin Hood, Markham, ON | White all purpose flour
Fleischmann’s Yeast, Lasalle, Quebec | Fleischmann’s quick rise instant yeast

2.2 Methods

2.2.1. Rheology Measurements

Viscosity was measured using a Synchro-electric Viscometer (D.W. Brookfield Ltd, Cooksville, ON), which measures the torque on a rotating spindle by means of a calibrated spring. Readings are made from a 0-100 scale on the dial. In this study spindle E and shear of 12 rpm was used and a constant temperature of 22°C was maintained. Viscosity in poise was calculated by multiplying the dial reading by the factor (3.90) corresponding to the viscometer spindle (E) and speed (12rpm) combination utilised (Brookfield Helipath Stand Spindle Factors, Stoughton MA,
USA). The fibres were mixed at a 1% concentration (1.5 g in 150 ml water). Measurements were taken at 15, 30, 45, 60, 90, 120, 180 min and 24hr.

2.2.2. Preparation of Fingerprick tubes

Fingerprick blood samples were collected in specially prepared tubes. To prepare the tubes, seven ml of distilled water was mixed with the contents of three potassium oxalate, sodium fluoride vacutainer tubes, 50μl of this solution was then pipetted into 7ml tubes (Sarstedt, St Leonard, Quebec). Seven ml of water was used together with 3 potassium oxalate tubes as it corresponds to the volume of blood intended for the glucose measurement tubes, i.e. assuming 1 drop of blood is 50μl, addition of 3-4 drops of blood to the 50μl of solution pipetted into the Sarsted tube will be at the same concentration of 7ml of blood in the 7ml vacutainer tube. The Sarstedt tubes were left to air dry for 24h before capping.

2.2.3 Blood Pressure Measurements

During the acute study all blood pressure measurements were made using a conventional mercury sphygmomanometer according to Joint National Council VI (NIH,1997) criteria where each measurement was repeated 3 times, separated by 2 minutes. However for the long-term study a digital blood pressure monitor was purchased, the OMRON HEM 907. This blood pressure monitor had been validated and satisfies the Association for the Advancement of Medical Instrumentation criteria for accuracy for a non-invasive blood pressure monitoring device (White and Anwar, 2001). The device can be programmed to take 3 blood pressure readings separated by a 1 minute interval. The advantages of using this machine are that blood pressure measurements can be made easily and are not influenced by the person who collects the data.

2.2.4 Body Fat Determination

The Futrex 5000 utilises near-infrared interactance to determine body composition. This method has been shown to correlate with hydrostatic densitometry
(Conway et al., 1984; Elia et al., 1990). The Futrex 5000 emits near-infrared light in the range of 938nm and 948nm, at these frequencies body fat absorbs the light and lean body mass reflects it. The Futrex measures the amount of light emitted from the light wand and reflected back into it, this provides an estimate of the distribution between body fat and lean body mass. The measurement is taken at the mid-point of the dominant bicep.

2.2.5 Measurement of circulating metabolites and hormones

2.2.5.1 Collection of blood samples

Blood samples were obtained at regular intervals using an indwelling catheter, kept patent with sterile 0.9% (w/v) saline and inserted into a vein in the cubical fossa (see study protocols for further details on blood sampling times). For the acute study each blood sample consisted of a total of 11ml of blood and collected into 2 mL sodium fluoride tubes for glucose and insulin, 4.5 ml EDTA tubes for NEFA and TAG, and 4.5 ml heparinised tubes to which aprotinin (SIGMA Chemical Company, St Louis, USA) was added at a concentration of 200 KIU/ml of blood, within 30 seconds of the sample being drawn. Heparinised samples were kept on ice at all times and centrifuged within 15min at 1500g for 10 min at 4 °C. For the long-term study additional blood samples were collected using standard vacutainers containing EDTA, a gel and clot activator, or sodium citrate. All samples were separated, frozen and stored at -70°C until analysed.

2.2.5.2 HbA1c measurement:

Prior to the start of the long-term study a method for determining HbA1c had to be chosen. There were two possibilities: the HPLC analysis performed by the laboratory at St Michael’s Hospital or the desktop method using the DCA 2000 made by Bayer. The DCA 2000 is a compact spectrophotometer that carries out all test functions of the system once the reagent cartridge has been inserted in the reaction chamber. The advantage of this machine is that the results are available within 6 minutes so that if there was a problem with the sample and a repeat sample was
required the subject would still be available. As the HbA1c levels would be the primary outcome of the long-term study this was an important advantage of the method. However, although the device had been validated in several studies (Arsie et al., 2000; Matteucci et al., 1998) an internal comparison was performed using 39 samples. The results were plotted using a Bland-Altman plot (Bland and Altman, 1995)

![Comparison of SMH vs DCA 2000 HbA1c Analysis (n=39)](image)

**Fig 2.1:** Bland-Altman plot of the difference of the measurements against the average of the two measurements of 39 samples

Although there was a highly significant correlation between the two methods ($p<0.002$) there were several outliers and a distinct positive bias at higher HbA1c levels. It was therefore decided to continue to analyse the samples the conventional way using the HPLC method (Cole et al., 1978). The interassay CV for the HPLC method was 2.4%.

2.2.5.3 Glucose analysis

2.2.5.3.1 Glucose analysis of whole blood:

Fingerprick blood samples were analysed for glucose using the YSI 2300 STAT PLUS (Yellow Springs, Ohio, USA). This machine uses a glucose oxidase method to analyse blood glucose levels. The probe of the machine is fitted with a
three layer membrane containing immobilized enzyme in the middle layer. When a blood sample is aspirated into the buffer-filled chamber, glucose diffuses through the membrane. On contact with the immobilised oxidase enzyme, it is rapidly oxidised, producing hydrogen peroxide:

\[
\text{Glucose} + O_2 \xrightarrow{GO} \text{Glucono-\textgreek{d}-lactone} + H_2O
\]

The hydrogen peroxide is then oxidised at the platinum anode, producing electrons. A dynamic equilibrium is achieved when the rate of H₂O₂ leaving the immobilized enzyme layer is constant, which is indicated by a steady state response. The electron flow is linearly proportional to the steady state H₂O₂ production and the rate at which H₂O₂ leaves the immobilised enzyme layer are constant.

The instrument was calibrated with a standard glucose solution prior to analysis of the samples using a solution of 10mmol/L glucose and this calibration was repeated every 5 samples. The inter-assay coefficients of variation for glucose were 3.3% and 1.8% at 3.9mmol/L and 14.4 mmol/L respectively.

2.2.5.3.2. Glucose analysis on plasma samples

Plasma glucose from the venous samples were analysed by the Banting and Best Core Laboratory (Toronto) again using a glucose oxidase method and a Cobas Integra Analyser (Roche Diagnostics). The interassay coefficients of variation were 3.5% and 1.7% at 4.0 mmol/L and 15.5 mmol/L respectively.

2.2.5.4. Analysis of insulin:

For the acute study the samples were analysed by the Banting and Best Core Laboratory using a double antibody radioimmunoassay (Livesey et al., 1980). In this analysis the insulin in the samples competes with a fixed amount of 125I-labeled insulin for the binding sites on the specific antibodies. Bound and free insulin are separated by addition of a second antibody immunoadsorbent followed by
centrifugation and decanting. The radioactivity in the pellet is then measured. The radioactivity is inversely proportional to the quantity of insulin in the sample. The inter assay coefficients of variation for the method at different levels were 7.2, 6.6 and 8.8% at insulin concentrations of 72, 316 and 753 pmol/L respectively. The lower detection limit of the assay was 22 pmol/L.

While the long term study was in progress the Banting and Best Lab changed the insulin analysis from radioimmunoassay to a electrochemiluminescence immunoassay (ECLIA) procedure. With this method the insulin in the samples is incubated with a biothylated monoclonal specific antibody and a monoclonal insulin-specific antibody labelled with ruthenium complex. Streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. These microparticles are then magnetically captured on the surface of the electrode. Application of a voltage to the electrode induces chemiluminescent emission which is measured by a photomultiplier. The inter assay CV is between 1.5-2.0%. The lower detectable limit of the test is 1.39 pmol/L.

Unfortunately one of the limitations of this analysis is haemolysis of the samples which interferes with the analysis. Our samples had been collected into fluoride oxalate tubes and haemolysis of the samples was quite common. This had never been a problem with the radioimmunoassay but the insulin results using this analysis on our samples were unacceptable with results ranging from -116 pmol/L to 655 pmol/L. Another kit (Mercodia Insulin Elisa, Uppsala, Sweden) which claimed that haemolysis would not interfere with the analysis was tried to see if better results could be obtained. When test runs were done with this kit using plasma samples to which haemoglobin had been added (Table 2.1), the results were very encouraging with only the higher levels of haemoglobin causing unacceptable decreases in insulin levels (table 2.1).
Table 2.1: Analysis of plasma insulin levels in plasma samples to which haemoglobin had been added at 5 different concentrations to estimate the amount of interference.

<table>
<thead>
<tr>
<th>Hgb g/L</th>
<th>Absorbance</th>
<th>Insulin mU/L</th>
<th>Insulin pmol/L</th>
<th>% change (decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well 1</td>
<td>Well 2</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.198</td>
<td>0.201</td>
<td>0.200</td>
<td>15.5</td>
</tr>
<tr>
<td>0.5</td>
<td>0.199</td>
<td>0.189</td>
<td>0.194</td>
<td>15.0</td>
</tr>
<tr>
<td>1</td>
<td>0.189</td>
<td>0.186</td>
<td>0.188</td>
<td>14.4</td>
</tr>
<tr>
<td>2</td>
<td>0.189</td>
<td>0.186</td>
<td>0.188</td>
<td>14.4</td>
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<tr>
<td>4</td>
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<td>0.178</td>
<td>0.179</td>
<td>13.6</td>
</tr>
<tr>
<td>6</td>
<td>0.164</td>
<td>0.170</td>
<td>0.167</td>
<td>12.4</td>
</tr>
</tbody>
</table>

Hgb - haemoglobin

However when samples from the long term study were analysed using the Mercodia ELISA kit, the samples which were badly haemolysed again gave negative insulin results. It was decided therefore to analyse insulin and glucose levels on samples which had been collected into tubes which had sodium citrate as the preservative. Unfortunately these samples were only available for the fasting levels and plans to analyse insulin levels on the oral glucose tolerances samples which had been collected at the beginning and end of each 3 months treatment period had to be abandoned.

2.2.5.5. Immunoassays for analysis GIP and GLP-1

2.2.5.5.1. Plasma GIP

Plasma GIP concentrations were measured using a double antibody radioimmunoassay developed by Dr. Linda Morgan and colleagues at the University of Surrey (Morgan et al., 1978). The actual analysis of both GIP and GLP-1 was kindly performed by Dr Kelly Johnston who at that time was a PhD student with Dr Morgan.

The antiserum was raised by immunising rabbits against porcine GIP conjugated to ovalbumin and crossreacts 100 % with human, rat and mouse GIP, exhibiting negligible cross reactivity with secretin, glucagon, GLP-1, VIP, pancreatic polypeptide, insulin. Radiolabelled GIP (125I) was prepared at the University of Surrey and was used as the tracer in this assay. Biosynthetic human GIP (Sigma-
Aldrich, Poole, Dorset) was used as the standard, which was diluted into charcoal-stripped serum (CSS), produced at the University of Surrey using Norit A charcoal and serum collected from fasted volunteers. Anti-rabbit Sac-Cell (IDS, Tyne and Wear, UK), a solid second antibody suspended in cellulose was used in the separation of the antibody-bound and free GIP. Each sample was counted against the standard curve in order to ascertain the concentration of GIP in each sample.

Low and high QC plasma samples were included at the beginning and the end of each assay. The intra-assay coefficient of variation for low and high quality control samples at 60 pmol/L and 383 pmol/L were calculated to be 12.08% (low QC) and 4.32% (high QC). The interassay coefficients of variation were 20.16% (low QC) and 7.89% (high QC).

2.2.5.5.2. Plasma GLP-1 analysis

Plasma GLP-1 concentrations were measured using radioimmunoassay methods already established at the University of Surrey (Elliott et al., 1993). Antiserum specific for the C-terminal amidated forms of the peptide was raised in rabbits against synthetic human GLP-1 (7–36) amide conjugated to bovine serum albumin. The percentage cross-reactivity of this antiserum is as follows: 100% cross-reactivity with GLP-1 from all species tested and is specific for the C-terminal end of GLP-1, requiring a C-terminal amide for cross-reactivity. Negligible cross-reactivity with GLP-1(7–37), GIP, secretin, glucagon, GLP-2, VIP, pancreatic polypeptide, motilin, somatostatin. Radiolabelled GLP-1 (7–36) amide (125I) was prepared at the University of Surrey and was used as the tracer in this assay. Biosynthetic human GLP-1 (Peninsula, St. Helens, USA) was used as the standard, diluted into CSS which was produced at the University of Surrey using Norit A charcoal and serum collected from fasted volunteers. Separation of antibody-bound and free antigen was achieved by the use of anti-rabbit Sac-Cell, a solid phase second antibody suspended in cellulose (IDS, Tyne and Wear, UK).

Low and high QC plasma samples were included at the beginning and the end of each assay. The intra-assay coefficients of variation for low and high quality control samples at 13 pmol/L and 39 pmol/L were calculated to be were 12.08% (low
QC) and 4.32% (high QC). The interassay coefficients of variation were 20.16% (low QC) and 7.89% (high QC).

2.2.5.6. Triacylglycerol (TAG) and Non-Esterified Fatty Acids (NEFA) Analysis:

Both TAG and NEFA from the day profile study described in Chapter 4, were analysed in Dr Morgan’s laboratory using the Alpha Wasserman SPACE centrifugal analyser.

2.2.5.6.1. TAG Analysis

TAG are triesters of glycerol with fatty acids. The method of TAG determination is based on the quantitative measurement of the glycerol liberated from the hydrolysis of the TAG. The test involves the following reactions:

\[
\text{Triacylglycerol} \xrightarrow{lipases} \text{glycerol + fatty acids}
\]

\[
\text{Glycerol + ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3-phosphate + ADP}
\]

\[
\text{Glycerol-3-phosphate} + \text{O}_2 \xrightarrow{\text{Glycerol-3-phosphate oxidase}} \text{dihydroxyacetone-phosphate} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{4 Aminoantipyrin} + \text{4-chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + \text{HCl} + 4\text{H}_2\text{O}
\]

The absorbance of the quinoneimine is measured bichromatically at 505 nm/692 nm and is directly proportional to the TAG concentration. Each kit (Ace Triglycerides Reagent, Alfa Wasserman, Woerden, The Netherlands) contained a buffer solution and enzyme reagent:

**Buffer reagent:**

- Pipes buffer: 40 mmol/L, pH 7.5
- 4-chloro-phenol: 5.0 mmol/L
Magnesium ions 5.0 mmol/L
Adenosine-5'-triphosphate 1.0 mmol/L
Lipases ≥150 U/ml
Peroxidase ≥0.5 U/ml
Glycerol kinase ≥0.4 U/ml
Sodium Azide 0.05%

The inter-assay coefficient of variation for the assay was 0.110.96 (low QC) and 5.6 (high QC).

2.2.5.6.2. NEFA Analysis

The NEFA analysis is based on the conversion of NEFA to their copper salts, which are extracted into an organic solvent. The salts are then complexed with a dye, which can be measured colorimetrically at 550nm:

\[
\text{NEFA} + \text{ATP} + \text{CoA} \rightarrow \text{Acyl CoA Synthetase} \rightarrow \text{CoA} + \text{AMP} + \text{PPI}
\]

\[
\text{Acyl CoA} + \text{O}_2 \rightarrow \text{Acyl CoA Oxidase} \rightarrow 2,3\text{-trans-Enoyl-CoA} + \text{H}_2\text{O}_2
\]

\[
2\text{H}_2\text{O}_2 + \text{TOOS} + \text{4-AA} \rightarrow \text{Peroxidase} \rightarrow \text{Purple adduct} + 4 \text{H}_2\text{O}
\]

4-AAP = 4-aminoantipyrine,
TOOS = N-ethyl-N-(2-hydroxy-3-sulphopropyl) m-toluidine,
PPI = pyrophosphate.

The Randox NEFA kit was used to analyse the samples in Dr Morgan’s laboratory. Each kit consisted of:

Buffer reagent:
Phosphate buffer 0.04 mol/L, pH 6.9
Magnesium Chloride surfactant 3 mmol/L
Enzymes/Coenzymes:
Acyl Coenzymes A synthetase ≥0.3 U/ml
Ascorbate oxidase ≥1.5 U/ml
Coenzyme A 0.9 mmol/L
ATP 5.0 mmol/L
4-aminoantipyrine 1.5 mmol/L

Enzyme diluent:
Phenoxyethanol surfactant 0.3%
Maleimide 10.6 mmol/L

A calibration curve was established for each new batch of reagents using the supplied 1 mmol/L standard. NEFA concentration could then be calculated using the formula: \( A_{\text{sample}} \times \text{Concentration of standard}/ A_{\text{standard}} \). The inter-assay coefficient of variation for the assay was 5.96 (low QC) and 7.15 (high QC).

2.2.5.7. Analysis of Total-cholesterol, LDL-Cholesterol, HDL-cholesterol, TAG and C-reactive protein

All plasma lipid and CRP analyses for the long-term study described in Chapter 7 were carried out at the J. Alick Little Lipid Research Laboratory, University of Toronto. Throughout the study period this laboratory maintained certification in part III of standardization for cholesterol, triacylglycerol and HDL cholesterol measurement under the National Heart, Lung, and Blood Institute, Centers for Disease Control Lipid Standardization Program (1982).

2.2.5.7.1 Total Cholesterol Measurement:

Total cholesterol was measured using the Bayer (Technicon) RA 1000 Chemistry Analyzer (Bayer, Germany) using the Technicon method No. SM4-0139D91, release date April 1991.

The principle of the method is that cholesterol esterase completely hydrolyses cholesterol esters to free cholesterol. The free cholesterol, in the presence of oxygen and cholesterol oxidase, produces hydrogen peroxide which in turn is used to form a quinoneimine dye. The concentration of the dye, measured at 500 nm is directly proportional to the cholesterol content in the sample. The inter-assay coefficient of variation for the assay were 0.69% (low QC) and 0.63% (high QC).
Reagents used were: Bayer Cholesterol Reagent: Product No. T01-1684-02 and Bayer Wetting Agent Product No. T01-1970-85.

2.2.5.7.2. TAG Measurement

TAG was measured using the Bayer (Technicon) RA 1000 Chemistry Analyzer (Bayer, Germany) using the Technicon Method No. SM4-0173D91, release date April 1991.

The analysis is based on the conversion of TAG to glycerol and free fatty acids by lipoprotein lipase and is the same as described earlier in section 2.2.5.6.1.

Reagents used were: Bayer Triglyceride reagent: Product No. T01-1868-02. The inter-assay coefficient of variation for the assay were 1.06% (low QC) and 0.90 (high QC).

2.2.5.7.3. HDL Cholesterol Measurement

HDL can be isolated (Warnick et al., 1982) in serum by selectively precipitating low density lipoproteins (LDL) and very low density lipoproteins (VLDL) using magnetically enhanced reagent containing dextran sulphate (50,000 MW) and magnesium chloride. Applying a magnetic field to the mixture pulls away all precipitated lipoproteins, leaving only HDL in the supernatant. Using conventional methods to measure cholesterol in the supernatant, the specific HDL cholesterol content of the serum can be assessed by multiplying the cholesterol result by 1.1.

Reagents used were: dextran sulfate (M 50,000 ±5000) (Dextralip 50, Warnick &Co, Issaquah, WA), MgCl₂H₂O reagent grade, sodium azide NaN₃, gentamicin sulfate, and chloramphenicol. The inter-assay coefficient of variation for the assay was 1.54%.

53
2.2.5.7.4. Calculation of LDL

LDL-cholesterol levels were estimated using the Friedewald equation (Friedewald et al., 1972):

$$LDL-\text{Chol} = \text{Total-cholesterol} - (\text{TAG}/2.2 + \text{HDL-cholesterol})$$

The equation is a valid estimation as long as the concentration of TAG is less than 4.5mmol/L (Friedewald et al., 1972).

2.2.5.7.5. C-Reactive Protein (CRP) Analysis

CRP was measured on the Behring Nephelometer BN 100 (Burladingen, Germany).

Polystyrene particles coated with monoclonal antibodies to CRP were agglutinated when mixed with samples containing CRP. The intensity of the scattered light in the nephelometer is proportional to the CRP concentration in the sample. Result were evaluated by comparison with a standard curve of known concentration (Rifai and Ridker, 2001).

The reagents used were: N Reaction buffer, product code: OUMS 61, N Latex CRP Mono, Product code: OQIY21, N Latex CRP Mono, Product code: OQIY13, N/T Rheumatology Control level 1, product code OQDB13, N/T Rheumatology Control level 2, product code OQDC13, N/T Apolipoprotein Serum Control, Product code: OUPH07, Reference range: less than 3.8mg/L, sensitivity 0.19 mg/L. The inter-assay coefficient of variation for the assay were 3.31% (low QC and 3.31% (high QC).
2.2.5.8. Estimation of LDL oxidation in serum:

Estimation of LDL oxidation was carried out in the laboratory of Dr David Jenkins. LDL oxidation in serum samples was estimated using the procedure by Ahotupa et al (Ahotupa et al., 1996). The first step in the estimation of LDL oxidation is the isolation of LDL by precipitation using buffered heparin. The precipitation buffer consisted of 0.064 M trisodium citrate, adjusted to pH 5.0, and contained 50,000 IU/L heparin. Prior to the precipitation, 50µl of EDTA solution was added to 0.5ml of the serum samples and the samples and precipitation reagents were allowed to equilibrate to room temperature. After the addition of 3.5ml buffer, the tubes were incubated for 10 min. The insoluble lipoproteins were then sedimented by centrifugation at 1000g (2100rpm) for 10 min. The pellet re-suspended in 0.5ml of saline. The level of LDL in the samples was estimated using a Sigma Cholesterol kit. LDL oxidation was estimated by the baseline levels of diene conjugation in lipid fraction of the LDL. Lipids were extracted from 100µl of the LDL suspension using a chloroform-methanol reagent, dried under nitrogen and then re-dissolved immediately in 900µl cyclohexane. Absorbance was read spectrophotometrically at 234nm. Conversion to mM was made using the molar extinction coefficient of 29.5 mM⁻¹. The CV was 4.4% and 4.5% for within and between-assay precision respectively.

2.3 Ginseng Analysis

Analysis of 4 different ginseng samples, using standard techniques, was performed by Dr Arnason at the University of Ottawa. A following HPLC-UV technique developed for the American Botanical Council Ginseng Evaluation Program (Fitzloff et al., 1998) was used. The levels of three 20(S)-protopanaxatriols (Rg1, Rf, Re) and four 20(S)-protopanaxadiols (Rb1, Rb2, Rc, Rd) were identified. The HPLC conditions included: chromatograph, Beckman HPLC system; column, reverse-phase Beckman ultrasphere C-18, 5 µm octadecylsilane, 250x4.6 mm column; mobile phase, de-ionised water and acetonitrile; flow rate, 1.3 ml/min; UV detection, a module 168 diode-array detector set at 203 nm. The ginsenoside standards for Rg1 and
Re were provided by Dr H Fong, University of Illinois and the Rf, Rb1, Rc, Rb2, Rd standards were provided by Indofine Chemical Co., Somerville, NJ, USA.

2.4 Data Handling and Statistical Analysis

2.4.1 Area calculation

Incremental areas under the curve were analysed geometrically using the method by Wolever et al (Wolever et al., 1991). The area under the curve is based on the blood glucose-response curve above the baseline only using the formula:

$$\frac{A}{2} + A + \frac{(B-A)}{2} + B + \frac{(C-B)}{2} + C + \frac{(D-C)}{2} + D + \text{etc}$$

A, B, C and D represent positive glucose increments, t is the time interval between blood samples.

2.4.2 Statistical Analysis

Number Cruncher Statistical Software (Kaysville, Utah) was used for all acute studies. For the long term study statistical analyses was performed using the SAS software (SAS version 8.2, 2001; SAS Institute). Details of analysis are described in each chapter.

2.4.3 Insulin sensitivity calculations:

2.4.3.1 Homeostasis Model Assessment (HOMA):

HOMA was calculated according to the formula: $22.5/\text{FPG} \times \text{FPI}$ (Matthews et al., 1985) were FPG is the fasting the plasma glucose value and FPI is fasting plasma insulin value.

2.4.3.2 Quantitative Insulin Sensitivity Index (QUICKI):

The modified QUICKI was calculated according to the formula $1/\log(\text{fasting glucose}) + \log(\text{fasting insulin}) + \log(\text{fasting NEFA})$ (Perseghin et al., 2001).
2.4.3.3. The insulin sensitivity index (ISI):

The ISI for whole body insulin sensitivity was calculated using OGTT plasma glucose (PG) and insulin (PI) outcome, according to the formula by Matsuda et al. (Matsuda and DeFronzo, 1999): 10 000 divided by the square root of ([FPG\times FPI] \times ([mean \ PG \times mean \ PI])), where PG is expressed in mg/dl (0.0551mmol/L) and PI in \mu U/ml (6pmol/L).
Chapter 3

SELECTION OF EFFECTIVE TREATMENT MATERIALS

3.1. Chapter Synopsis

Neither ginseng or konjac-mannan are standardised products, therefore variability in their composition can occur. Consequently physiological effects can vary depending on the preparation used. To ensure that both the ginseng and konjac-mannan to be used in the studies were physiologically active, preliminary tests were performed:

3.1.1. Selection of effective Konjac-mannan fibre

Effectiveness of viscous fibre in lowering post-prandial glycaemia has been related to viscosity, therefore rheology measurements were taken to select the most viscous of 3 different konjac-mannan fibres available. All 3 fibres increased viscosity of the liquid over time, the fibre which increased the viscosity to the greatest extent over the first 3 hours was selected to be used in future experiments.

3.1.2. Selection of effective American ginseng preparation

Prior studies have shown that the ginseng extracts supplied by the Chai-Na-Tai company lowered post-prandial glycaemia both in healthy (Vuksan et al., 2000a; Vuksan et al., 2001a; Vuksan et al., 2000d) and diabetic volunteers (Vuksan et al., 2000a; Vuksan et al., 2001b). However as there were insufficient capsules left for further studies the company supplied a new batch of the extract. In addition, capsules containing whole ground ginseng root were also supplied. To compare these new products with the previous ones, the ginsenoside content was analysed. All samples showed the typical ginsenoside profile associated with American ginseng, however the replacement extract contained substantially lower ginsenoside levels than the other samples. The whole ground ginseng root was therefore selected for use in future experiments.
3.2. Introduction:

Both ginseng and konjac-mannan have been shown to reduce post-prandial glycaemia, however effectiveness of these materials is dependent on different factors:

Viscous fibres have been shown to reduce the rate of glucose absorption (Jenkins et al., 1978) and lower serum cholesterol (Anderson et al., 2000; Brown et al., 1999; Olson et al., 1997). Panel III of the US National Cholesterol Program for the first time, specifically mentioned the term “viscous” to define more precisely the sub class of soluble fibres which lowers serum lipids(NCEP, 2001). It has been clearly demonstrated that viscosity is an important attribute of fibre required to flatten the post-prandial glycaemia (Jenkins et al, 1978), although the data are not as clear in relation to the need for high viscosity in order to lower serum cholesterol (Blake et al., 1997). Commercially available sources of viscous fibre may vary greatly in viscosity and this could account for the lack of effect on post-prandial glycaemia of viscous fibres in some studies (Simpson et al., 1981; Williams and James, 1979).

As described in the literature review, it has been suggested that the glycosidal saponins called ginsenosides present in the ginseng root are the active components of ginseng. As with viscosity in commercial fibre sources so also there is great variability in the level of ginsenosides in commercial ginseng sources and preparations. Interspecies variation in ginsenosides has been reported to be as high as 15-fold in dried powder and 36-fold in liquid preparations (Harkey et al., 2001).

A simplified method to assess viscosity was used to select the konjac-mannan fibre. This method has proved to be a useful way to select the most physiologically active viscous fibres in previous studies (personal communication, V.Vuksan). A Synchro-electric viscometer was used to assess the relative in vitro viscosity of three sources of konjac mannan to determine the most viscous source. Ginsenoside content in four different types of ginseng was analysed and the ginseng with the highest total ginsenoside level was selected for use in the study.
3.3 Selection of effective Konjac-mannan fibre:

3.3. Methods

Viscosity measurements were taken of three different konjac mannan fibres (see Chapter 2, section 2.1.2) and compared to the wheat bran control (AACC certified hard red spring wheat). The fibres were mixed at a 1% concentration (1.5 g in 150 ml water). Viscosity was measured using the Synchro-electric Viscometer as described in the methods section. In this study spindle E and shear of 12 was used and a constant ambient temperature of 22°C (room temperature) was maintained. Measurements were taken at 15, 30, 45, 60, 90, 120, 180 min and 24hr. Results are tabulated in Table 3.1 and graphed in figure 3.1 and represent the mean of two experiments.

3.3.2. Results

There was no change in viscosity in the solution containing wheat bran. All konjac mannan fibres increased the viscosity of the solution over time. At 30min KJM1 had the highest viscosity when compared to KJM2 and KJM3 (296 vs 35 and 228 poise respectively). Peak viscosity was reached at 60 min by KJM1, 24hr by KJM3 and 120min by KJM3. KJM 1 achieved the highest viscosity absolute level of viscosity throughout the 24h measured.
Table 3.1: Viscosity measurements of wheat bran and three konjac mannan fibres.
Mean of two measurements

<table>
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<tr>
<th>Time (min)</th>
<th>15</th>
<th>30</th>
<th>45</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
<th>24hr</th>
</tr>
</thead>
<tbody>
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<td>2</td>
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<td>2</td>
<td>4</td>
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<td>2</td>
</tr>
<tr>
<td>KJM 1</td>
<td>214</td>
<td>296</td>
<td>298</td>
<td>293</td>
<td>291</td>
<td>274</td>
<td>279</td>
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</tbody>
</table>

*KJM=konjac-mannan fibre; +viscosity (poise) was calculated by multiplying the dial reading by the factor (3.90) corresponding to the viscometer spindle (E) and speed (12rpm) combination utilised (Brookfield Helipath Stand Spindle Factors, Stoughton MA, USA)

Fig 3.1: Comparison of viscosity levels in poise over 24h of wheat bran and three different konjac-mannan fibres.

3.3.3. Discussion

Viscosity can be altered by a variety of factors including temperature, molecular weight, and pH (Yoshimara and Nishinari, 1999). This experiment uses a simplified protocol to screen the different fibres, it does not try to mimic
physiological conditions such as body temperature and pH. The temperature was chosen for convenience and because konjac-mannan fibre is generally very stable at temperatures below 70°C. Similarly, we could have chosen to vary the pH levels to correspond to levels found in the stomach and small intestine, however, as pH levels ranging from 2.5-6 also do not change konjac viscosity significantly, this was not deemed to be necessary (manufacturer's specifications, Fibre Tech Co.). In addition, previous experiments utilising this simple experimental protocol correlated well with physiological effectiveness of the fibre. Therefore, the fibre which attained the highest viscosity in the shortest time, and maintained this over 3h, was selected for use in further studies.

3.4. Selection of effective American ginseng preparation:

3.4.1. Methods

Analysis of 4 different ginseng samples, using standard techniques, was performed by Dr Arnason at the University of Ottawa. The following HPLC-UV technique, developed for the American Botanical Council Ginseng Evaluation Program (Fitzloff et al., 1998), was used. Details of the method have been described earlier. The levels of three 20(S)-protopanaxatriols (Rg₁, Rf, Re) and four 20(S)-protopanaxadiols (Rb₁, Rb₂, Rc, Rd) were identified. The four samples of American ginseng analysed included the original extract (A) used in earlier, acute, studies (Vuksan et al., 2000a; Vuksan et al., 2001a; Vuksan et al., 2000c; Vuksan et al., 2000d), the extract (B) used in a previous, long term study, CNT 2000 (Vuksan et al., 2001b), the new batch of extract (C), and a whole ground ginseng (D). All samples were provided by the Chai-Na-Ta Company. Results are presented in Table 3.2.
### Table 3.2: Analysis of four samples of American ginseng:

<table>
<thead>
<tr>
<th>Ginsenosides (% w/w)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(20S)-protopanaxadiols:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rb₁</td>
<td>1.53</td>
<td>1.34</td>
<td>0.65</td>
<td>1.71</td>
</tr>
<tr>
<td>Rb₂</td>
<td>0.06</td>
<td>0.08</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>Rc</td>
<td>0.24</td>
<td>0.46</td>
<td>0.11</td>
<td>0.84</td>
</tr>
<tr>
<td>Rd</td>
<td>0.44</td>
<td>0.62</td>
<td>0.12</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>(20S)-protopanaxatriols:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rg₁</td>
<td>0.10</td>
<td>0.13</td>
<td>0.08</td>
<td>0.17</td>
</tr>
<tr>
<td>Re</td>
<td>0.83</td>
<td>0.91</td>
<td>0.67</td>
<td>1.46</td>
</tr>
<tr>
<td>Rf</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total ginsenosides</strong></td>
<td>3.21</td>
<td>3.54</td>
<td>1.66</td>
<td>5.20</td>
</tr>
<tr>
<td><strong>Ratios:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPD:PPT</td>
<td>2.44</td>
<td>2.40</td>
<td>1.20</td>
<td>2.19</td>
</tr>
<tr>
<td>Rb₁:Rg₁</td>
<td>15.30</td>
<td>10.31</td>
<td>8.13</td>
<td>10.06</td>
</tr>
<tr>
<td>Rb₂:Re</td>
<td>0.25</td>
<td>0.17</td>
<td>0.18</td>
<td>0.19</td>
</tr>
<tr>
<td>Rg₁:Re</td>
<td>0.12</td>
<td>0.14</td>
<td>0.11</td>
<td>0.11</td>
</tr>
</tbody>
</table>

A - ginseng used in prior studies (Vuksan et al., 2000a; Vuksan et al., 2001a; Vuksan et al., 2000c; Vuksan et al., 2000d); B - ginseng extract used in the long term study with type 2 diabetes, (Vuksan et al., 2001b); C - new batch of ginseng to replace extract B; D - whole ground ginseng. PPD = (20S)-protopanaxadiols; PPT = (20S)-protopanaxatriols
3.5 Chapter Discussion

The composition of all 4 samples are consistent with *Panax quinquefolius* L. Presence and/or absence of various ginsenosides and their ratios are used in the authentication of the ginseng species. A ratio of Rb1: Rg1>3 (Awang, 2000; Ma et al., 1996) and ratios of Rg1:Re<1 and Rb2:Rc<1 have been shown to be indicative of *Panax quinquefolius* L. (Chan et al., 2000; Wang et al., 1999). However the presence or absence of the ginsenoside Rf is the most significant marker, as it is absent in *Panax quinquefolius* altogether (Awang, 2000; Chan et al., 2000; Li et al., 2000; Ma et al., 1996). These criteria were met by all ginseng samples. Ginseng samples A and B showed similar ginsenosides profiles with totals of 3.21 and 3.54% w/w respectively. Sample D had the highest total level of ginsenosides (5.2% w/w). However sample C, with a total ginsenoside level of 1.66 % w/w, had 47% less total ginsenosides, and a 51% lower PPD:PPT ratio, the differences mainly due to lower PPD levels.

The two samples, A and B, which have been shown to be effective in improving glycaemic control in type 2 diabetes (Vuksan et al., 2000a; Vuksan et al., 2001a; Vuksan et al., 2001b), had similar ginsenoside profiles. Although the new ginseng extract C was supposed to be a replacement for B, it had a much lower total ginsenoside level than the original extract (1.66% vs 3.54%).

The lower level of ginsenosides in sample B stresses the importance of quality control and analysis not only of different samples of ginseng, but also of different batches. As sample D contained the highest level of ginsenosides, it was decided to use this ginseng in future studies.
Chapter 4

DAY PROFILE STUDY IN TYPE 2 DIABETES

4.1 Chapter Synopsis

With the selection of a potentially physiologically active ginseng and fibre samples, the in vivo testing could now be undertaken. In previous in vivo studies ginseng (AG) had been shown to reduce postprandial blood glucose levels through raising insulin levels, while konjac mannan fibre (KJM) had been shown to increase insulin sensitivity.

4.1.1 Study Design:

The study employed a two-by-two factorial, partially blinded, controlled randomised cross-over design. Subjects were given a standard breakfast on four different occasions to which had been added either: 1. placebo, 2. ginseng, 3. konjac mannan or 4. konjac mannan and ginseng. In addition a standard lunch (second meal) was consumed on all four days. Blood samples were collected over 7.5 hours.

4.1.2 Study Hypothesis

A. During the first 4 hours of the test (after breakfast):

1. Ginseng will:
   - increase GIP and GLP-1

2. Konjac mannan fibre will:
   - suppress postprandial NEFA levels and reduce postprandial GIP and GLP-1 levels

3. Ginseng and konjac fibre, when used in combination will:
   - reduce postprandial glycaemia, and suppress postprandial NEFA levels
B. During the second 3.5 hours of the test (after lunch):
Ginseng and konjac mannan will:

- improve postprandial glycaemia and insulinaemia

4.1.3 Methods

Ten individuals with type 2 diabetes (age 66±6y, BMI 28.7±9.2 kg/m², A1c 6.8±1.1%) participated in the study. Four sets of breakfasts, matched for energy and fibre content, containing 50g available carbohydrate from Ensure™, were administered in random order. 1: Control (7g of wheat bran); 2: AG (3g of American ginseng + 7g of wheat bran); 3: KJM (4g of Konjac mannan); 4: AG+KJM (3g of American ginseng + 4g of Konjac mannan). This was followed by a second meal, a standard lunch, 4h later. Blood samples were obtained at -30, 0, 15, 30, 60, 90, 120, 150, 180, 240, 270, 300, 360 and 420 min.

4.1.4 Results

At 15 min the incremental plasma glucose levels after AG were significantly lower than the control (p<0.02). Incremental area calculations of the second meal ie 240-420min showed a reduction in glucose area after KJM and AG+KJM when compared to control and AG alone (p<0.03). Incremental insulin levels were significantly lower after AG+KJM at 30 min when compared to AG: (p<0.03). At 120min insulin levels were significantly lower after both KJM and AG+KJM than AG (p<0.03), and at 420min AG was significantly higher than control, KJM and AG+KJM (p<0.01). There were no significant differences in insulin areas or in the insulin sensitivity index. Incremental GIP levels were decreased after KJM and AG+KJM at 30min when compared to AG (p<0.04). At 90 and 120min, GIP levels after KJM were significantly lower than both control and AG (p<0.02), at 150min KJM was significantly lower than AG (p<0.04). The Control incremental GIP area was significantly higher than the KJM GIP area, while the area for AG was significantly higher than both the KJM and AG+KJM areas (p<0.01). There were no differences in the incremental GLP-1 response between control and AG, however
after KJM, GLP-1 was significantly lower at 60 and 90 min when compared to control (p<0.04 and p<0.03 respectively). After AG+KJM, GLP-1 was lower at 90 min when compared to control (p<0.03). The total areas for KJM and AG+KJM were significantly lower than the control (p<0.08), there were no significant differences between the incremental areas. There were no significant differences between the meals in NEFA, TAG, blood pressure levels, and satiety.

4.1.6 Conclusion:

The ginseng used in this study showed a small but significant reduction in postprandial glycaemia, the lack of a more sustained effect on both glucose and insulin might have been due to its composition. This emphasises the importance of the development of a standardised, effective ginseng preparation. Konjac-mannan decreased postprandial insulin, GLP-1 and GIP levels. Lack of effect on glucose might be due to lack of the viscosity of the meal. Using konjac-mannan and ginseng in combination did not, in this study, improve the metabolic profile more than when konjac-mannan was used alone.
4.2 Introduction

Both ginseng and konjac mannan have been shown to reduce postprandial glycaemia (Kim et al., 1996; Vuksan et al., 2000a)(fig 4.1 and 4.2), however the mechanisms of action to achieve this reduction is different for each substance (see Literature Review). Consequently, it has been postulated that using ginseng and konjac-mannan in combination, in the treatment of diabetes, may have additive beneficial effects. This would be similar to the current practice of combining oral hypoglycaemic agents in the treatment of diabetes.

Fig 4.1: Comparison of incremental changes in glycaemia in type 2 diabetes between American ginseng and a matched corn flour placebo administered either 40 minutes before or together with a 25g g oral glucose challenge. Incremental glycaemia at individual time points with different lowercase letters are significantly different (p<0.05). From: (Vuksan et al., 2000a).
In order to study the possible additive effects of ginseng and konjac mannan a study was designed which would compare the acute metabolic effects of ginseng and konjac mannan alone and in combination in type 2 diabetes. From previous studies with ginseng we would expect a reduction of postprandial glycaemia together with an increase of postprandial insulin levels (Vuksan et al., 2000a; Vuksan et al., 2001b). It is not known if ginseng affects postprandial levels of NEFA, TAG, GIP and GLP-1. Both GIP and GLP-1 are incretins which increase the early insulin response to a mixed meal and as ginseng increases postprandial insulinemia, we hypothesised that this increase may be modulated through GIP and/or GLP-1.

Viscous fibres have been shown to flatten the postprandial glucose, insulin, GIP, and enteroglucagon levels (Jenkins et al., 1977; Morgan et al., 1979). In addition, viscous fibre administration has also been associated with sustained suppression of NEFA (Jenkins et al., 1980). It was expected that konjac mannan would elicit a similar postprandial metabolic profile. It is not known what effect, if any, konjac mannan would have on GLP-1.

Previous studies with guar gum have shown that addition of guar to a first meal, flattens the glycaemic and insulinaemic response not only to the first meal but
also to a fibre free second meal (Jenkins et al., 1980). In part this effect was attributed to the suppression of NEFA (Jenkins et al., 1980). It is not known whether konjac-mannan will have the same effect as guar on the second meal or whether the presence of ginseng will affect the metabolic response to subsequent meals. This might be of interest with respect to timing of administration i.e. whether it is necessary to take the fibre/ginseng with each meal or if it can be taken less frequently. The acute study therefore proposed to study the metabolic response to ginseng and konjac mannan not only over breakfast but also over a subsequent, standard second meal.

Doses of ginseng and konjac used in the study were based on previous work carried out in our laboratory (Vuksan et al., 2000a). Therefore 3g of Ginseng or corn flour (control) capsules were administered 30min prior to the breakfast. Capsules were administered before breakfast because previous work showed that ginseng only lowered postprandial glycaemia in healthy volunteers when given 30-40min before a meal (Vuksan et al., 2001a; Vuksan et al., 2000c). Although timing of the ginseng dose may not be as important in diabetic subjects (Vuksan et al., 2000c)(Fig 4.1), it was decided nevertheless to follow the healthy volunteer protocol and capsules were therefore given thirty minutes before breakfast. Similarly the dose of konjac mannan was based on our previous experience with konjac fibre in acute tests and we estimated that addition of 4g of Konjac-mannan (3.5g of soluble fibre) to 50g available carbohydrate will reduce the postprandial glycaemia in type 2 diabetes, approximately 45-50% (Vuksan et al., 1992)(Fig 4.2.). To simulate a meal, Ensure™ (Abbott Laboratories Limited, Saint-Laurent, Quebec, Canada), a liquid meal replacement, was used instead of glucose. Ensure™ was chosen as it was felt to be more physiological representative of a meal than glucose, it contains protein and fat in addition to carbohydrate. A further advantage of Ensure™ is that, although it constitutes a meal, it can be consumed in a relatively short time and the konjac mannan fibre can easily be mixed into it. This is important as viscous fibre has been shown to be more effective when mixed intimately with the carbohydrate portion of the meal (Wolever et al., 1979).

Blood samples were analysed for plasma glucose, insulin, GIP, GLP-1, NEFA and TAG. In addition, blood pressure measurements were taken throughout the day as both ginseng (Stavro et al., 2000b) and konjac (Vuksan et al., 1999b) have been
shown to reduce blood pressure in long-term studies, and there is some suggestion that certain ginsengs may effect blood pressure acutely (Stavro et al., 2002).

4.2.1 **Hypothesis**

A. During the first 4 hours of the test (after breakfast):

1. **Ginseng will**:
   - □ Reduce postprandial glycaemia
   - □ Increase postprandial insulinaemia
   - □ Increase glucose-dependent insulino tropic secretagogue hormones: GIP and GLP-1

2. **Konjac mannan fibre will**:
   - □ Decrease postprandial glycaemia and insulinaemia
   - □ Suppress postprandial NEFA levels
   - □ Reduce postprandial GIP and GLP-1 levels

3. **Ginseng and konjac fibre, when used in combination will**:
   4. Reduce postprandial glycaemia, and
   5. Suppress postprandial NEFA levels
   6. Not change postprandial insulinaemia

B. During the second 3.5 hours of the test (after lunch):

**Ginseng and konjac mannan will**:
- □ Improve postprandial glycaemia and insulinaemia
4.3 Research Design and Methods

4.3.1. Volunteers:

4.3.1.1. Power Calculation

It was assumed that the tests were conducted at a significance level of alpha=0.05 and the power to detect a significant difference was 90%. Based on previous studies with Konjac and ginseng, it was assumed that the expected standard deviation for plasma glucose (area under the curve) would be 73 min.mmol/L and for insulin, at 15 min, 32 pmol/L. The minimal detectable significant differences for these parameters were 110 min.mmol/L and 55 pmol/L respectively. From this set of assumptions, the minimum sample size calculated was 12. Assuming a 15-20% attrition rate, the final sample size was calculated to be 14.

4.3.1.2 Subjects

Thirteen volunteers with type 2 diabetes completed the study. However 3 subjects had to be eliminated because of a change in oral hypoglycaemic medication during the study period (subjects #1, #4 and #15). Therefore a total of 10 subjects were included in the final data analysis, age (mean±SD) 66±6 y, BMI 28.7±9.2 kg/m², duration of diabetes 8.4±5y, HbA1c 6.8±1%, 2 treated with diet alone, 8 treated with oral hypoglycaemic agents. Exclusion criteria included: diagnosis of type 2 diabetes of less than 6 months duration, A1c>9%, use of ginseng within the past 2 months, women taking hormone replacement therapy; gastrointestinal disease; cigarette smoking or heavy alcohol intake (>2 drinks/day). All volunteers gave written informed consent to participate in the study. The study was approved by the St Michael's Hospital Ethics Committee.

4.3.2 Protocol

Participants attended the Risk Factor Modification Centre at St Michael's Hospital on four separate occasions following a 10-12 hour overnight fast. They were
instructed to maintain the same dietary and exercise patterns the evening before each test and ensure adequate carbohydrate intake. Participants were asked to complete a questionnaire detailing pre-session information regarding their diet and lifestyle patterns and record the previous night’s dietary intake. Subjects adhered to their usual medication regime during each test day.

The study utilized a two-by-two factorial, controlled randomised crossover, partially blinded, design. Four sets of breakfasts were administered in random order. All breakfasts used Ensure™ (Abbott Laboratories Limited, Saint-Laurent, Quebec, Canada) as the source of carbohydrate. Ginseng and control (corn flour, International Nutrition Ltd, Markham, Ontario) capsules were administered 30 min before breakfast while konjac mannan and the fibre control (certified hard red spring wheat bran, American Association of Cereal Chemists, St Paul, USA) were mixed into the Ensure™. The four breakfasts which were administered were:

1. **Control**: Ensure + control capsules + wheat bran,
2. **KJM**: Ensure + control capsules + konjac mannan fibre
3. **AG**: Ensure + Ginseng capsules + wheat bran
4. **AG+KJM**: Ensure + Ginseng capsules + konjac mannan fibre.

Lunch was a standard meal of soup, sandwich and fruit (see tables 4.4 and 4.5). The test days were randomised for each subject and were scheduled at least one week apart. The total test day spanned 7.5 hours. Blood samples were obtained using an indwelling catheter, kept patent with sterile 0.9% saline and inserted into a vein in the cubical fossa. Blood pressure was measured using a conventional mercury sphygmomanometer according to Joint National Council VI (NIH,1997) criteria where each measurement was repeated 3 times, separated by 2 minutes. Subjects were asked to record their satiety levels throughout the day using a bipolar scale ranging from -3 (extremely hungry), 0 (neutral), to +3 (uncomfortably full). The palatability of the meals was recorded on a scale from 1 to 10, where 1 was “dislike extremely”, 5 “neutral” and 10 “delicious”. At each visit, weight was measured using a beam scale and total body fat was measured with the Futrex 5000 (Futrex Inc. Gaithersburg, USA), using infrared technology. Table 4.1 shows the times when meals and measurement were taken.
Table 4.1: Schedule of measurements taken during the day profile study

<table>
<thead>
<tr>
<th>Measurement</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>6h</th>
<th>7h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous Blood</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Blood</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Satiety</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Palatability</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsules</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meals</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body Fat</td>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.3.3 Materials and Meals

Control capsules, containing corn flour (International Nutrition Ltd, Markham, Ontario), were developed to balance the macronutrient content of the ginseng capsules (Chai-Na-Ta Corporation, Langley, British Colombia) (see table 4.2). Each dose consisted of 3g (6 capsules) of either ginseng or corn flour. All breakfasts contained 50g carbohydrate supplied by 356g of Ensure™ (see table 4.3 for nutritional composition) and 200ml of water. Wheat bran (Certified Hard Red Wheat Bran, American Association of Cereal Chemists, St Paul, Minnesota, USA) was used as the fibre control for konjac mannan (FiberJohnson, Toronto, On, Canada)(Table 4.3). Due to the nature of the fibres it was not possible to blind the subjects to the type of fibre they received.
Table 4.2: Macronutrient content of 1 gram of either corn flour (control) or American ginseng

<table>
<thead>
<tr>
<th></th>
<th>Corn Flour (Control)</th>
<th>American Ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>3.51</td>
<td>3.44</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>0.73</td>
<td>0.57</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>0.039</td>
<td>0.013</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>0.069</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Macronutrient analysis determined by the Association of Official Analytical Chemists methods for macronutrients analysis (1980).

Table 4.3: Composition of Ensure™, Konjac and Wheat bran fibre used in tests meals

<table>
<thead>
<tr>
<th></th>
<th>Ensure™ (356g)</th>
<th>Konjac** (4g)</th>
<th>Wheat Bran** (7g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (g)</td>
<td>50</td>
<td>NA</td>
<td>0.9</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>12.4</td>
<td>NA</td>
<td>1.1</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>8.8</td>
<td>NA</td>
<td>0.3</td>
</tr>
<tr>
<td>Fibre - total</td>
<td>0</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>- soluble</td>
<td>NA</td>
<td>NA</td>
<td>0.2</td>
</tr>
<tr>
<td>- insoluble</td>
<td>NA</td>
<td>NA</td>
<td>3.3</td>
</tr>
</tbody>
</table>

* Nutrient information obtained from manufacturer: Abbott Laboratories Limited, Saint-Laurent, Quebec, Canada
** Macronutrient analysis determined by the Association of Official Analytical Chemists methods for macronutrients analysis (1980).

After the -30min blood sample, subjects swallowed the control or ginseng capsules with 200ml of water. At 0min another blood sample was drawn, the fibre was mixed with the Ensure™ and subjects were instructed to consume the mixture over 5 min. Four hours after the start of breakfast a standard lunch was offered, macronutrient composition and details of the meals are given in tables 4.4 and 4.5. In addition each subject had a choice of beverage (coffee, tea or water) at lunch. The type and amount of beverages selected were kept constant for each test day.
Table 4.4: Macronutrient composition of test meals

<table>
<thead>
<tr>
<th>Meal</th>
<th>Energy</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Fat</th>
<th>Fibre in meal</th>
<th>Added Fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast:</td>
<td>1487 KJ</td>
<td>12g</td>
<td>50g</td>
<td>13g</td>
<td>0g</td>
<td>3.5g**</td>
</tr>
<tr>
<td>(Ensure™)</td>
<td>(354kcal)</td>
<td>(14%)</td>
<td>(55%)</td>
<td>(32%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch*</td>
<td>2284 KJ</td>
<td>24 g</td>
<td>75g</td>
<td>19g</td>
<td>5g</td>
<td>0g</td>
</tr>
<tr>
<td></td>
<td>(544kcal)</td>
<td>(17%)</td>
<td>(53%)</td>
<td>(30%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* beverage not included; ** fibre from either wheat bran or konjac mannan fibre

Table 4.5: Foods consumed during breakfast and lunch on each test day

<table>
<thead>
<tr>
<th>Time</th>
<th>Test 1 (Control)</th>
<th>Test 2 (KJM)</th>
<th>Test 3 (AG)</th>
<th>Test 4 (AG+KJM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsules</td>
<td>-30' Corn flour + 250 ml water</td>
<td>Corn flour + 250 ml water</td>
<td>Ginseng + 250 ml water</td>
<td>Ginseng + 250 ml water</td>
</tr>
<tr>
<td>B’fast</td>
<td>0' Ensure™ (356g)+ wheat bran (7 g) + 200ml water</td>
<td>Ensure™ (356g)+ Konjac (4g) + 200ml water</td>
<td>Ensure™ (356g)+ wheat bran + 200ml water</td>
<td>Ensure™ (356g)+ Konjac + 200ml water</td>
</tr>
<tr>
<td>Lunch</td>
<td>240' Lipton’s instant vegetable soup</td>
<td>Lipton’s instant vegetable soup</td>
<td>Lipton’s instant vegetable soup</td>
<td>Lipton’s instant vegetable soup</td>
</tr>
<tr>
<td></td>
<td>3 sl white bread (84g)</td>
<td>3 sl white bread (84g)</td>
<td>3 sl white bread (84g)</td>
<td>3 sl white bread (84g)</td>
</tr>
<tr>
<td></td>
<td>2 tsp Canola margarine (8g)</td>
<td>2 tsp Canola margarine (8g)</td>
<td>2 tsp Canola margarine (8g)</td>
<td>2 tsp Canola margarine (8g)</td>
</tr>
<tr>
<td></td>
<td>3 sl cheese (57g)</td>
<td>3 sl cheese (57g)</td>
<td>3 sl cheese (57g)</td>
<td>3 sl cheese (57g)</td>
</tr>
<tr>
<td></td>
<td>Tomato slices (80g)</td>
<td>Tomato slices (80g)</td>
<td>Tomato slices (80g)</td>
<td>Tomato slices (80g)</td>
</tr>
<tr>
<td></td>
<td>Apple slices (100g)</td>
<td>Apple slices (100g)</td>
<td>Apple slices (100g)</td>
<td>Apple slices (100g)</td>
</tr>
<tr>
<td></td>
<td>Beverage of choice</td>
<td>Beverage of choice</td>
<td>Beverage of choice</td>
<td>Beverage of choice</td>
</tr>
</tbody>
</table>

Abbreviations: AG - American ginseng, KJM - konjac mannan fibre
4.3.4 **Hormone and Metabolite Measurements**

Each blood sample consisted of a total of 11ml of blood and collected into sodium fluoride tubes for glucose and insulin, EDTA tubes for NEFA and TAG, and heparinised tubes to which aprotinin (SIGMA Chemical Company, St Louis, USA) was added at a concentration of 200 KIU/ml of blood, within 30 seconds of the sample being drawn. Heparinised samples were kept on ice at all times and centrifuged within 15min at 1500g for 10 min. All samples were separated, frozen and stored at -70°C until analysis.

The glucose concentration of each sample was determined by a glucose oxidase method (Kadish and Hall, 1965). A double antibody radioimmunoassay was used to determine insulin concentrations (Livesey et al., 1980) (see Chapter 2). The inter-assay coefficients of variation for glucose was 3.3 and 1.8% at 3.9 and 14.4 mmol/L respectively; for insulin 7.2, 6.6 and 8.8% at 72, 316 and 753 pmol/L respectively.

The whole body insulin secretion index (ISI) was calculated using the method described by (Matsuda and DeFronzo, 1999), using the formula:

\[
10,000 \\
ISI = \frac{1}{\sqrt{\text{FPG} \times \text{FPI} \times \text{mean PG} \times \text{mean PI}}}
\]

where plasma glucose (PG) and fasting plasma glucose (FPG) are expressed in mg/dl (0.0551 mmol/L) and plasma insulin (PI) and fasting plasma insulin (FPI) in μU/ml (6pmol/L). The mean PG and PI are calculated using the results from the first 2 hours of the OGTT.

GIP and GLP-1 were analysed using in-house radioimmunoassay methods (Elliott et al., 1993; Morgan et al., 1978). NEFA and TAGs, were analysed using standard colorimetric analytical techniques (Randox Colorimeter, Randox Laboratories Inc, Crumlin, UK)(See Chapter 2).
The NEFA analysis is based on the conversion of NEFA to their copper salts, which are extracted into an organic solvent. The salts are then complexed with a dye, which can be measured calorimetrically. TAG analysis is based on the quantitative measurement of the glycerol liberated enzymatically from the hydrolysis of TAG. See Chapter 2 for more detailed descriptions of analytical methods.

4.3.5 Statistical analysis

Plasma glucose, insulin, GIP, and GLP-1, NEFA and TAG were graphed, and the positive incremental area under the curve (AUC) was calculated geometrically for each participant (Wolever et al., 1991) (see Chapter 2 for details of area calculation). AUC was calculated separately for the first 4 hours and the last 3.5 hours. Between the four types of test meals, differences in AUC were assessed by one-way repeated measures ANOVA, and adjusted for multiple pair-wise comparisons with the Newman Keuls procedure. The same statistical method was used to assess the independent effect at the individual time points. As the major effect seemed to reside with konjac mannan, a test of contrast was also performed. This allowed comparison of those tests which contained konjac with those that did not. Body fat and weight was analysed using two-way ANOVA to test for differences between treatments and to test for treatment-by-time interaction. All statistical analyses were performed using the Number Cruncher Statistical System 2000 software (NCSS statistical software, Kaysville, UT, USA). Results are expressed as mean ± SEM and significant at p< 0.05.

4.5 RESULTS

4.4.1 Fasting Levels

There were no statistical differences between the fasting levels of any of the metabolites measured (table 4.6).
Table 4.6: Fasting levels at -30min and 0min for 10 subjects with diabetes before taking 4 different test meals. There were no statistically significant differences between fasting levels.

<table>
<thead>
<tr>
<th></th>
<th>Fasting at -30min</th>
<th>Fasting at 0min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>AG</td>
</tr>
<tr>
<td>Glucose mmol/L</td>
<td>8.7±0.7</td>
<td>9.1±1.0</td>
</tr>
<tr>
<td>Insulin pmol/L</td>
<td>62±17</td>
<td>66±17</td>
</tr>
<tr>
<td>NEFA mmol/L</td>
<td>0.58±0.24</td>
<td>0.61±0.21</td>
</tr>
<tr>
<td>TAG mmol/L</td>
<td>1.77±0.15</td>
<td>2.05±0.3</td>
</tr>
<tr>
<td>GIP pmol/L</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>GLP-1 pmol/L</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Syst BP mmHg</td>
<td>120±3</td>
<td>122±4</td>
</tr>
<tr>
<td>Diast BP mmHg</td>
<td>74±2</td>
<td>73±2</td>
</tr>
</tbody>
</table>

4.4.2 Weight and Body Fat

There were no significant changes in total body weight and body fat throughout the test period (Table 4.7).

Table 4.7: Total body weight and body fat values for 10 subjects during the day profile study (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Control +Wheat Bran</th>
<th>Ginseng + Wheat Bran</th>
<th>Control + Konjac</th>
<th>Ginseng + Konjac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>88.4±16.4</td>
<td>88.3±16.5</td>
<td>88.2±16.5</td>
<td>88.5±16.4</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>28.8±7.3</td>
<td>29.1±7.1</td>
<td>28.5±6.7</td>
<td>29.2±7.0</td>
</tr>
</tbody>
</table>
4.4.3 Glucose and Insulin

At 15 min the incremental plasma glucose levels after AG were significantly lower than the control: 0.8±0.3 vs. 1.76±0.3 mmol/L respectively (p<0.02) (Fig 4.3). There were no significant differences in the areas under the curve between 0-240min. Incremental area calculations of the second meal ie 240-420min, and using 240min values as the baseline, showed a reduction in glucose area after KJM and AG+KJM when compared to control and AG alone (p<0.03) (Fig 4.4 and Table 4.8).

Fig 4.3: Incremental plasma glucose day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM) followed by a standard lunch at 240min. Results are expressed as mean ± SEM; * AG significantly lower than control (p<0.02).
Fig 4.4: Incremental areas under the curve for glucose in 10 subjects with type 2 diabetes over the 2nd meal after control, ginseng (AG), konjac-mannan (KJM) and the combination of ginseng and konjac mannan (AG+KJM). Results are expressed as mean ± SEM. Bars with different letters are significantly different (p<0.05).

Incremental insulin levels were significantly lower after AG+KJM at 30 min when compared to AG: 82.4±25.3, 127.7±40.1pmol/L respectively (p<0.03). At 120min both KJM and AG+KJM were significantly lower than AG (173.9±43.6, 167.5±43.7 and 254.7±65.2pmol/L respectively) (p<0.03), and at 420min AG (154.2±41.2pmol/L) was significantly higher than control, KJM and AG+KJM (166.7±45.1, 138.8±32.7 and 154.2±41.2pmol/L respectively)(p<0.01)(Fig 4.5). There were no significant differences in insulin areas (Table 4.8) or in the insulin sensitivity index.
Fig 4.5: Incremental plasma insulin day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM) followed by a standard lunch at 240min. Results are expressed as mean ± SEM. Time points with different letters are significantly different.

4.4.3.1. Contrast Test

When a contrast test was performed ie a comparison of the glucose and insulin levels between the tests which contained konjac mannan (KJM and AG+KJM) and those that did not (Control and AG), only the incremental glucose area under the curve was again significant for the 2nd meal. The same test applied to the insulin results showed consistent significantly lower insulin levels at 30, 90 and 120min (P<0.05). The incremental insulin area after the konjac mannan containing meals was significantly decreased over the first 4 hours of the test (p<0.01). The incremental insulin area over the 2nd meal was not significantly different. The same test applied to ginseng containing meals did again show a significant lower glucose value at 30min and at 300min (p<0.04) but no differences in incremental areas.
4.4.4 **NEFA and TAG**

There were no significant differences between the meals in NEFA or TAG levels. (Fig 4.6 and 4.7).

**Fig 4.6:** Incremental plasma NEFA day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM) followed by a standard lunch at 240min. Results are expressed as mean ± SEM.

**Fig 4.7:** Incremental plasma TAG day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM) followed by a standard lunch at 240min. Results are expressed as mean ± SEM.
4.4.5 GIP and GLP-1

Only the first 4 hours of the day profile were analysed for GIP and GLP-1. As starting GIP levels for subject #9 were not available, this subject was excluded from the analysis. Incremental GIP levels were decreased after KJM and AG+KJM at 30min when compared to AG (174±31, 149±34 and 174±31 pmol/L respectively) (p<0.04). At 90 and 120min (134±22 and 92±18pmol/L), KJM was significantly lower than both control (217±35 and 157±31pmol/L) and AG (223±44 and 181±43pmol/L) (p<0.02), at 150min KJM (76±19pmol/L) was significantly lower than AG (131±33pmol/L) (p<0.04), (Fig 4.8). The incremental GIP area for KJM was significantly lower than both the Control and AG areas (23286±3708, 34953±6010 vs 37830±6809pmol/L respectively) (p<0.01) and the AG+KJM area was significantly lower than the AG area (26184±5771 vs 37830±6809pmol/L) (Fig 4.9).

GLP-1 was significantly reduced at 60 and 90min after KJM when compared to control (10.4±6.6, and 2.6±4.4 vs 30.9±11 and 25.0±10pmol/L, p<0.04 and p<0.03 respectively). GLP-1 was significantly lower at 90min after AG+KJM when compared to control (13.0±3.9 vs 26±4.4pmol/L, p<0.03). There were no differences in the incremental GLP-1 response between AG and control (Fig 4.10).

Although there were no significant differences in starting levels, when absolute GLP-1 levels were used in the statistical analysis the differences were more marked: when compared to the control values KJM was significantly lower at 60, 90, 120, and 180 min (p<0.05) and after AG+KJM at 60, 90,150 and 180min (p<0.05). Similarly the total areas for KJM and AG+KJM (4127±1041 and 3755±668min.pmol/L) were significantly lower than the control (6583±1137 min.pmol/L, p<0.008), whereas with the incremental areas, only the konjac was significantly different from the control (Fig 4.9 and Table 4.8).

4.4.5.1. Contrast Test

When incremental areas of GIP and GLP-1 of the konjac containing meals (KJM and AG+KJM) where compared to the non-konjac containing meals (Control and AG), konjac containing meals were significantly lower than those which did not contain konjac (1585±427 vs 3242±681min.pmol/L, p<0.01), there was no difference
in incremental areas between the ginseng containing meals and those which did not contain ginseng.

Fig 4.8: Incremental plasma GIP day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM). Results are expressed as mean ± SEM. Time points with different letters are significantly different.

Fig 4.9: GIP Incremental areas for control, ginseng, konjac mannan and the combination of ginseng and konjac. Bars with different letters are significantly different (p<0.01)
AG: American ginseng, KJM: Konjac-mannan fibre, AG+KJM: American ginseng and konjac combination
Fig 4.10: Incremental plasma GLP-1 day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannann (KJM) or a combination of ginseng and konjac mannann (AG+KJM). Results are expressed as mean ± SEM. Time points with different letters are significantly different.

Fig 4.11: Total and incremental areas of GLP-1 for control, ginseng, konjac mannann and the combination of ginseng and konjac. Bars with different letters are significantly different.

AG: American ginseng, KJM: Konjac-mannann fibre, AG+KJM: American ginseng and konjac combination
4.4.6 Blood Pressure

Systolic and diastolic blood pressure results are presented in Fig 4.12 and 4.13. The systolic blood pressure shows the greatest fall on the KJM test meal, however it also has the highest starting value 129±5mmHg compared to 119±4 mmHg although this was not statistically significantly different. Statistical analysis using two-way Anova, did not show any significant differences.

![Day Profile - Change in Systolic BP](image)

**Fig 4.12:** Incremental Systolic blood pressure day profile results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM). Results are expressed as mean ± SEM.

![Day Profile Study - Change in Diastolic BP](image)

**Fig 4.13:** Incremental Diastolic blood pressure results of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM). Results are expressed as mean ± SEM.
4.4.7 Satiety, Palatability and Eating Time

There were no significant differences in satiety, palatability or eating time between the different meals over either breakfast of lunch.

![Day Profile - Satiety](image)

**Fig 4.14:** Total satiety score over breakfast and lunch of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM). Results are expressed as mean ± SEM.

![Palatability of Breakfast and Lunch](image)

**Fig 4.15:** Total palatability scores for breakfast and lunch of 10 subjects with type 2 diabetes after either control, ginseng (AG), Konjac mannan (KJM) or a combination of ginseng and konjac mannan (AG+KJM). Results are expressed as mean ± SEM.
Table 4.8: Means of incremental areas under the curve (AUC) for glucose, insulin, GIP, GLP-1, and TAG over breakfast (0-240min) and the second meal (240-420min). AG=American Ginseng, KJM=konjac mannan fibre, N/A=not analysed.

### Incremental AUC 0-240min

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>AG</th>
<th>KJM</th>
<th>AG+KJM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min.mmol/L)</td>
<td>757±112</td>
<td>708±113</td>
<td>820±96</td>
<td>759±95</td>
</tr>
<tr>
<td>Insulin</td>
<td>3311±6659</td>
<td>34612±7629</td>
<td>27267±6240</td>
<td>27708±7321</td>
</tr>
<tr>
<td>(min.pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIP</td>
<td>3495±6016</td>
<td>37830±6809</td>
<td>23286±3708</td>
<td>26184±5771</td>
</tr>
<tr>
<td>(min.pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1</td>
<td>6016±959</td>
<td>6808±694</td>
<td>3709±684</td>
<td>5771±352</td>
</tr>
<tr>
<td>(min.pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>92±17</td>
<td>86±21</td>
<td>90±17</td>
<td>77±14</td>
</tr>
<tr>
<td>(min.mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Incremental AUC 240-420min

<table>
<thead>
<tr>
<th>Test</th>
<th>Control</th>
<th>AG</th>
<th>KJM</th>
<th>AG+KJM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(min.mmol/L)</td>
<td>389±47</td>
<td>410±75</td>
<td>233±39</td>
<td>238±51</td>
</tr>
<tr>
<td>Insulin</td>
<td>2498±7346</td>
<td>31524±12548</td>
<td>23286±8697</td>
<td>20066±4999</td>
</tr>
<tr>
<td>(min.pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIP</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>(min.pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>(min.pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TAG</td>
<td>60±13</td>
<td>54±14</td>
<td>48±11</td>
<td>52±9</td>
</tr>
<tr>
<td>(min.mmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.5 Chapter Discussion

Previous acute studies with ginseng have shown postprandial glucose lowering with concomitant increases in postprandial insulin levels (Vuksan et al., 2000a) as well as decreases in blood pressure (Stavro et al., 2000a). However, the physiological effectiveness of ginseng can be variable and is dependent on the ginseng preparation (Sievenpiper et al., 2003). In this study, the ginseng preparation used, was only shown to decrease glucose levels at 15 min. Insulin levels tended to be higher both during the first and second meal but this did not reach significance when compared to the control. Several factors might be responsible for these results: First, the study may be underpowered after having to eliminate 3 subjects, secondly there is still controversy regarding what constitutes the physiologically active component(s) of ginseng. The ginseng used in the study was selected on the basis of total ginsenosides, however the ratio of key ginsenosides might be of greater importance (Hong et al., 2000). Low protopanaxadiols (PPD) to protopanaxatriols (PPT) ratios have been shown to inhibit non-insulin stimulated glucose uptake in adipocytes at the highest dose of 10,000 μg/ml and significantly inhibited insulin stimulated glucose uptake compared to control in a dose related manner (Hong et al., 2000). This particular batch of ginseng had a slightly lower ratio of PPD: PPT than a previous, more effective, batch. Alternatively, other components such as peptidoglycans, polysaccharides and quinquefolians also present in ginseng might be responsible for the glucose lowering seen with some batches of ginseng.

As ginseng has been shown to increase postprandial insulin levels (Vuksan et al., 2001b) it was hypothesised that this was achieved by increased levels of the insulinotropic hormones GLP-1 and GIP, however no such increases were seen in this study. Another mechanism of action must therefore be responsible for the increase in insulin levels seen after ginseng administration.

Konjac-mannan fibre too has been shown to decrease postprandial glucose levels but, unlike ginseng, this is accompanied by decreased postprandial levels of insulin. In this study, although no effect on glucose was seen, the postprandial insulin levels were decreased significantly. Similarly GLP-1 and GIP were decreased after
the meals containing konjac-mannan. Previous studies with guar showed reductions in postprandial GIP levels (Ellis et al., 1995; Jenkins et al., 1988; Morgan et al., 1979). These results therefore support the hypothesised slow release mechanism of action of viscous fibres.

Guar has been shown to also decrease the postprandial glycaemic and insulinaemic response to a second, standard meal, after the fibre was administered in the first meal. It was expected that konjac-mannan would have a similar effect. It was not known if ginseng would affect the metabolic response to a second meal. In this study konjac-mannan did decrease the incremental glucose area of the second meal, however this effect was mainly due to higher starting levels present before the second meals was consumed. These higher levels seen 4 hours after consuming a viscous-fibre rich meal, although not significantly different, again support the slower release carbohydrate hypothesis where one of the beneficial effects of fibre is ascribed to its ability to smooth out the glucose and insulin curves thereby preventing the sharp increase and concomitant undershoot of both glucose and insulin levels seen after fibre free meals.

In spite of the differences in insulin levels there were no significant differences in NEFA levels. NEFA levels did not go back to baseline, which might explain the lack of effect as NEFA levels may have already been maximally suppressed.

There was a tendency for TAG levels to be higher after the control meal over the first 2 hours but this only reached significance when incremental levels were calculated from -30min rather than 0min and might be therefore be related to starting levels.

Although konjac mannan affected postprandial insulin, GIP and GLP-1 levels there was no effect on postprandial glucose levels. Similar reductions in GIP and GLP-1 have been observed without affecting postprandial glucose levels after coffee or chlorogenic acid administration (Johnston et al., 2003). This might be a reflection of a relatively small gut effect and that plasma glucose levels are less sensitive to small changes in gut hormones than plasma insulin.
The general lack of effect of konjac-mannan in this study on glucose levels might also be due to the mode of administration. Subsequent tests showed that addition of konjac-mannan to Ensure did not result in the expected increase of viscosity of the liquid (see Chapter 5). The lack of viscosity of the meal could explain the lack of effect on glucose. In addition, it is possible that viscosity of the meal developed after ingestion and that the reduced levels of insulin, GIP and GLP-1 might be due an increase of viscosity lower down in the gastrointestinal tract.

Although shown in previous studies, (Stavro et al., 2002; Stavro et al., 2000b) (Vuksan et al., 1999b) in this study, no effect of either ginseng or konjac-mannan was seen on blood pressure. Again, effectiveness of this particular ginseng preparation might have been responsible for the lack of effect. Konjac-mannan has been shown to reduce blood pressure in longer term trials (Vuksan et al., 1999b), however acute reductions have not been reported. It is possible blood pressure reductions seen with konjac mannan might be the result of a general improvement in metabolic control rather than a direct, acute effect.

It has been suggested that fibre may play an important role in weight maintenance through increasing satiety (Blundell and Burley, 1987). The proposed mechanisms of action are delayed gastric emptying and intestinal transit time and/or reduction of postprandial glucose, insulin and GI peptide and hormone levels. Some studies have reported increases in satiety and/or suppression of appetite with soluble fibre (Kovacs et al., 2002; Pasman et al., 1997) but results vary (Ellis et al., 1981; Heini et al., 1998). One of the confounding factors might be GLP-1, which promotes satiety (Verdich et al., 2001), but which is generally reduced after the ingestion of fibre rich meals. In this study there were no differences in perception of satiety. Factors which may have been responsible for this result, may have been the reduced levels of GLP-1, the lack of gelling of the konjac mannan and the insensitivity of the bi-polar scale.

Although it had been expected that the combination of konjac-mannan and ginseng would further improve the metabolic response to a meal, in this study no additive effect was seen. The contrast tests performed on the data indicate the major
effective component was the konjac mannan fibre, therefore the lack of additive effect seen here may have been due to the overall small physiological activity of the ginseng preparation used in this study. Further studies will need to be undertaken to study possible additive effects.

**4.6 Conclusion**

The ginseng used in this study showed a small but significant reduction in postprandial glycaemia, the lack of a more sustained effect on both glucose and insulin might have been due to its composition. This emphasises the importance of the development of a standardised, effective ginseng preparation. Konjac-mannan decreased postprandial insulin, GLP-1 and GIP levels. Lack of effect on glucose might be due to lack of the viscosity of the meal. Using konjac-mannan and ginseng in combination did not, in this study, improve the metabolic profile more than when konjac-mannan was used alone.
Chapter 5

SUPPLEMENTARY STUDIES TO EXPLORE RESULTS FROM ACUTE STUDY

5.1. Chapter Synopsis

Studies were initiated to explore reasons for the failure of konjac and ginseng to exert the expected effect on circulating glucose and insulin levels seen in prior studies.

First the viscosity measurements were repeated with the konjac fibres to test whether the viscosity of the three konjac fibres maintain the same relationship when mixed with Ensure™ instead of water.

Secondly, \textit{in vivo} tests were undertaken to test, in healthy volunteers, whether the konjac when taken with Ensure™, showed the same lack of effect as seen with the Day Profile.

Thirdly, test meal studies were undertaken to compare the ginseng used in the day profile with a ginseng preparation which was known to be effective in lowering postprandial blood glucose levels. Test meals were given with and without konjac to evaluate again the effect of the combination of ginseng and konjac on postprandial glucose.

5.1.1 Results:

Rheology:

1. \textbf{Viscosity of Three Konjac Fibres when Mixed with Ensure™}: when konjac was mixed with Ensure the expected rise in viscosity was not observed.

\textit{In Vivo}:

2. \textbf{Postprandial Blood Glucose Response to Two Konjac Fibres} showed a significant 29 and 32\% reduction in incremental areas when konjac was added to Ensure™ (p<0.01).
3. **Postprandial Blood Glucose Responses to Three Ginsengs With or Without Konjac Fibre.** The ginseng used in the acute study, again showed no beneficial effect on blood glucose. The ginseng which had previously shown to be effective, significantly reduced the incremental glucose level at 90min ($p<0.05$) and reduced the AUC by 24% when compared to control but this did not reach significance. Addition of konjac did not show any additional benefit. Two way ANOVA analysis of konjac containing meals vs non-konjac containing meals showed a AUC reduction of 14% ($p<0.02$) by konjac.

5.1.2 **Conclusion**

Despite the use of viscous konjac fibre and high ginsenoside containing ginseng, no significant effect on post-prandial glucose or insulin levels were seen in subjects with type 2 diabetes (chapter 4). Supplementary studies in healthy volunteers also showed a relative lack of effect on post-prandial glycaemia and indicate that further studies are required to determine the optimal dose, source and nature of the supplements to maximise the effectiveness of konjac and ginseng for use in further studies.
5.2. Introduction

Effectiveness of viscous fibre has been related to its ability to increase the viscosity of the intra-luminal contents of the small intestine (Ellis et al., 1995). Prior to the acute study the relative viscosity of three konjac-mannan fibres had been determined to select the most viscous of the three fibres available. However in this determination the fibres were mixed with water, in the acute, *in vivo* test, the fibres were mixed with the meal replacement Ensure™. As the post-prandial glucose reductions by konjac-mannan were less than expected it was hypothesised that the ability of the konjac-mannan fibre to form a viscous gel in Ensure™ was reduced. To test this hypothesis, the viscosity experiment performed in Chapter 3 was repeated using Ensure™ as the base rather than water.

Although viscous fibre lowers post-prandial glycaemia when incorporated into solid mixed meals (Ellis et al., 1981; Jenkins et al., 1976; Kovacs et al., 2001; Kovacs et al., 2002) the effects are generally larger when the fibre is incorporated into liquid test meals such as water (Jenkins et al., 1978) or soup (Wolever et al., 1979). The results from the acute study and *in vitro* results were therefore unexpected. Additional *in vivo* studies with the konjac and Ensure™ combination were carried out because it was not understood whether the effect was due to the presence of diabetes or whether it was truly because the fibre is ineffectual when mixed with Ensure™. The two fibres (KJM1 and KJM 3), which achieved peak viscosity in the shortest time in the first rheology experiment, were used for *in vivo* testing.

In the acute day profile study carried out in chapter 4, the effect of ginseng on post-prandial glucose and insulin levels was also less than expected. It was therefore decided to repeat the study in healthy volunteers using the ginseng from the acute study (whole root ginseng) and compare it with a ginseng extract (CNT2000) which had been shown to be effective in previous studies in diabetic subjects (Vuksan et al., 2000a; Vuksan et al., 2001a). A third ginseng (Mary ginseng) was added to allow further comparison.
To evaluate whether addition of konjac to ginseng had an additive effect on blood glucose, all test meals were given either with or without konjac mannan fibre (see table 5.2).

5.3. **In Vitro Experiment: Rheology: Viscosity of Three Konjac Fibres when Mixed with Ensure™**

5.3.1 **Methods**

The earlier rheology experiment was repeated (see Chapter 3) using the same methods and materials with the exception that 150ml of Ensure™ was substituted for 150ml of water.

5.3.2 **Results**

The viscosity results are tabulated in table 5.1. The peak viscosity of the wheat bran was 6 poise, which is similar to the earlier results where the peak viscosity of wheat bran was 8 poise (table 3.1). However the viscosity of the konjac fibres this time, did not go higher than 14 poise compared to 246-335 poise when the fibres were mixed with water (Fig 5.1).

<table>
<thead>
<tr>
<th>Table 5.1: Viscosity measurements of wheat bran and 3 different konjac fibres with Ensure™ as the medium.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
</tr>
<tr>
<td>Wheat Bran</td>
</tr>
<tr>
<td>KJM 1</td>
</tr>
<tr>
<td>KJM 2</td>
</tr>
<tr>
<td>KJM 3</td>
</tr>
</tbody>
</table>

+Viscosity (poise) was calculated by multiplying the dial reading by the factor (3.90) corresponding to the viscometer spindle (E) and speed (12rpm) combination utilised (Brookfield Helipath Stand Spindle Factors, Stoughton MA, USA).
The results were unexpected, as the viscosity barely changed when the konjac fibre was added to the Ensure. The reason for this is not clear. According to manufacturers specifications konjac fibre forms a very stable gel under most conditions. They state that gelling of the fibre is not affected by other ingredients including milk proteins, and is constant with a pH between 4-10. However hydration and dispersion are key factors (Ellis and Morris, 1991), both of which might have been affected by the Ensure. The fibre was hand mixed into the Ensure, and although no clumps were visible, it is possible that the fibre was not dispersed sufficiently. Due to the high concentration of protein, fat, glucose and minerals in Ensure, it is possible that the water present is not available to hydrate the konjac fibre sufficiently to form a gel.

The lack of viscosity observed might in part explain the negative results observed in the day profile experiment. To confirm this, in vivo experiments in healthy volunteers were carried out.
5.4. *In Vivo Studies*

Two different studies were performed in healthy volunteers, the first ("konjac experiment") tried to evaluate whether it was the presence of diabetes which made the konjac ineffective in lowering postprandial glycaemia in the acute experiment. The second study ("ginseng experiment") compared the ginseng used in the acute study with a previously shown, effective ginseng. In addition this study also evaluated the effect of adding konjac to ginseng on blood glucose in healthy volunteers.

5.4.1. Methods

5.4.1.1. Power Analysis

The sample size was determined by power analysis. It was assumed that the tests were to be conducted at a significance level of alpha=0.05 and the power to detect a significant difference was 90%. Based on previous studies with konjac and ginseng, it was assumed that the expected standard deviation for the 90min area under the curve for plasma glucose was 60 min.mmol/L and the minimal detectable significant differences 72 min.mmol/L. From this set of assumptions, the minimum sample size calculated was 9 for both studies. Assuming a 15% attrition rate, the final sample size was calculated to be a minimum of 10.

5.4.1.2. Volunteers

1. Konjac experiment: Eleven healthy volunteers participated in the study and all completed the study protocol (4 men, 7 women, age (mean ±SD) 36 ±13 y., body mass index 23.5±3.4kg/m²).

2. Ginseng experiment: Ten volunteers were recruited for the study but two volunteers were unable to finish the test series, due to time constraints. Due to the limited supply of CNT2000 we were unable to enrol further subjects. Therefore eight healthy volunteers participated in the study (4 men, 4 women), age (mean ±SD) (28.4±12.0 y, BMI 23.4±3.9 kg/m²).

The studies were approved by the Ethics Committee of St. Michael’s Hospital. Informed written consent was obtained from all volunteers.
5.4.1.3. Protocol

The studies had 3 and 8 treatment segments for the konjac and ginseng experiments respectively and were given in a randomised order. Tests were performed at the Clinical Nutrition and Risk factor Modification Centre of St Michael’s Hospital. The standard fingerprick protocol was followed for both studies (see Chapter 2). In the konjac experiment, after a fasting finger-prick blood sample was obtained, KJM1, KJM3 or wheat bran was mixed with the Ensure™. Subjects were instructed to consume the Ensure™ over a 5 min period. For the ginseng experiment, a fasting finger-prick blood sample was obtained (-40min), after which the subjects swallowed the capsules with 200 ml of water. After 40 min, an additional fasting sample was obtained (0min) after which the subjects consume the Ensure™ and fibre mixture over a 5 min period. Immediately after the Ensure™ and fibre mixture was consumed, subjects consumed an additional 200 ml water (table 5.2).

5.4.1.4. Test Meals

All test meals consisted of 50 g available carbohydrate from 365g Ensure™ (Ross Products Division, Abbott Laboratories, Saint-Laurent, Canada) to which in the konjac experiment, 3.5g of fibre was added either as 7g wheat bran (control) or 4g of KJM1 or KJM3 was added. After the Ensure™ and fibre mixture was consumed, subjects drank 250 ml water. In the ginseng experiment, the three ginseng products used were: 1) Whole root ginseng (“Premium North American Ginseng “ (Chai-NaTai, NF Formulas Inc, Canada) which was the ginseng used in the acute study; 2) CNT 2000 (Chai Na Tai, NF Formulas Inc, Canada) an extract of ginseng, which had been shown previously to be effective and finally 3) “Mary ginseng” (Mary Ginseng House, Toronto, Canada) a locally produced, whole ground, ginseng. Each dose consisted of 3 grams (6 capsules) of either corn flour or ginseng, 200ml of water was given to swallow the capsules. The fibre used was either 7g of wheat bran (control) or 4g of KJM1 and was mixed with the Ensure just prior to consumption. Immediately after consuming the Ensure and fibre mixture, subjects consumed an additional 200ml of water (table 5.2).
Table 5.2: Ginseng experiment: Description of the 8 Test meals of the ginseng experiment comparing the effect of 3 different ginsengs with or without added konjac mannan fibre.

<table>
<thead>
<tr>
<th></th>
<th>Capsules</th>
<th>Ensure™</th>
<th>3.5g Fibre Source</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>C+WB</td>
<td>Control</td>
<td>356 g</td>
<td>7g of Wheat bran</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>C+KJM</td>
<td>Control</td>
<td>356 g</td>
<td>4 g of Konjac</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>CNT+WB</td>
<td>CNT 2000</td>
<td>356 g</td>
<td>7g of Wheat bran</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>CNT+KJM</td>
<td>CNT 2000</td>
<td>356 g</td>
<td>4 g of Konjac</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>MG+WB</td>
<td>Mary Ginseng</td>
<td>356 g</td>
<td>7g of Wheat bran</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>MG+KJM</td>
<td>Mary Ginseng</td>
<td>356 g</td>
<td>4 g of Konjac</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>G+WB</td>
<td>Whole root ginseng</td>
<td>356 g</td>
<td>7g of Wheat bran</td>
<td>200ml+200ml</td>
</tr>
<tr>
<td>G+KJM</td>
<td>Whole root Ginseng</td>
<td>356 g</td>
<td>4 g of Konjac</td>
<td>200ml+200ml</td>
</tr>
</tbody>
</table>

C=control capsules, WB=wheat bran, KJM=konjac mannan, CNT=ginseng capsules containing CNT2000 ginseng extract, MG=ginseng capsules containing ginseng from Mary Ginseng, G=whole root ginseng capsules. *Water – 200ml of water was consumed with the capsules at –40min and again at 0min immediately after the Ensure was consumed.

5.4.1.4. Sample Analysis

The blood glucose samples were analysed using a glucose oxidase method (Yellow Spring Instruments, 2300 Stat glucose/L-lactate analyser, model 115)(see Chapter 2).

5.4.1.5. Statistical Analysis

The results are expressed as means ± SEM. The incremental areas under the blood glucose response curve (0 to 90min), ignoring the area below the fasting level, were calculated geometrically (Wolever et al, 1991). A two-factor repeated measures ANOVA was used to assess differences in glucose levels between treatments. Between the meals, differences in AUC were assessed by one-way repeated measures ANOVA, and adjusted for multiple pair-wise comparisons with the Newman Keuls procedure. In all cases, differences were considered statistically significant if p<0.05. A further 2-way ANOVA was performed on the ginseng study to compare the glucose response to konjac containing meals with those without konjac.
5.4.2 Results

Konjac experiment: A repeated measures ANOVA over the time course of the experiment showed a significant difference in absolute glucose values between treatments (p<0.01). A comparison of individual time points showed that at 15 and 30min both KJM1 and KJM3 were significantly lower than the wheat bran (p<0.02), and at 45min KJM3 was significantly lower than the wheat bran (p<0.04) (Fig 5.2). The areas under the curve (AUC) for KJM1 and KJM3 were (Mean±SEM) 79.7±17.8 and 83.8±20.8 min.mmol/L respectively; both were significantly lower than the AUC for wheat bran of 117.7±18.1 min.mmol/L (p<0.01) (Fig 5.3). This represents respectively a 32 and 29% reduction in the incremental areas for KJM 1 and 3 when compared to the control.

![Incremental Glucose](image)

Fig 5.2: Incremental glucose levels in 11 healthy subjects after 356g Ensure mixed with either 7g wheat bran (control), or 4g of two konjac-mannan fibres with different viscosities (KJM1 and KJM3). Points with different letter are significantly different.
Fig 5.3: Incremental areas under the glucose tolerance curve after 356g Ensure mixed with either 7g of wheat bran (control), or 4g of two konjac-mannan fibres with different viscosities (KJM1 and KJM3). Bars with different letters are significantly different, p< 0.01.

Ginseng experiment: A repeated measures ANOVA over the time course of the experiment showed no significant differences in absolute glucose values between treatments. A comparison of individual time points showed that at 90min CNT2000 was significantly lower than all other values (p<0.05) (Fig 5.4). There were no significant differences in the areas under the curve (Table 5.3, Fig 5.5). Comparing all the meals containing konjac with those which did not, showed significant differences in glucose concentrations and treatment (p<0.03). The AUC for konjac containing meals was reduced by 14% (p<0.01) when compared to the non konjac containing meals.
Table 5.3: Incremental glucose areas under the curve of three different ginseng preparations with or without konjac mannan fibre, tested in 8 healthy subjects

<table>
<thead>
<tr>
<th>Test Meal</th>
<th>AUC (min.mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C+WB</td>
<td>135.1 ± 17.2</td>
</tr>
<tr>
<td>C+KJM</td>
<td>102.7 ± 18.1</td>
</tr>
<tr>
<td>G+WB</td>
<td>144.2 ± 28.6</td>
</tr>
<tr>
<td>G+KJM</td>
<td>127.2 ± 26.7</td>
</tr>
<tr>
<td>CNT+WB</td>
<td>102.4 ± 15.6</td>
</tr>
<tr>
<td>CNT+KJM</td>
<td>116.2 ± 16.7</td>
</tr>
<tr>
<td>MG+WB</td>
<td>136.7 ± 19.9</td>
</tr>
<tr>
<td>MG+KJM</td>
<td>102.3 ± 15.4</td>
</tr>
</tbody>
</table>

C=control capsules, WB=wheat bran, KJM=konjac mannan fibre, G= whole ground root ginseng capsules, CNT=CNT2000 ginseng capsules, MG= “Mary Ginseng” capsules.

Incremental Blood Glucose

Fig 5.4: Incremental glucose levels of three different ginseng preparations with or without konjac mannan fibre, tested in 8 healthy subjects. C=control capsules, WB=wheat bran, KJM=konjac mannan fibre, G= whole ground root ginseng capsules, CNT=CNT2000 ginseng capsules, MG= “Mary Ginseng” capsules. *p<0.05
Fig 5.5: Glucose AUC (90min) results of three different brands of American ginseng taken with or without konjac fibre. C=control capsules, WB=wheat bran, KJM=konjac mannan fibre, G=whole ground root ginseng capsules, CNT=CNT2000 ginseng capsules, MG="Mary Ginseng" capsules. Mean + SEM, n=8

When comparing the previously used, effective ginseng (CNT2000) with the new ginseng (whole root) used in the acute study, there were no significant differences in incremental blood glucose values when compared to control (Fig 5.6). Although the 90min incremental area under the curve for CNT 2000 was lower (102.4 ± 15.6 min.mmol/L) than either the control (135.1 ± 17.2 mmol/L) or the whole root ginseng (144.2 ± 28.6 mmol/L) it did not reach significance (p=0.16).
Fig 5.6: Incremental blood glucose levels after control (C+WB), previously used ginseng (CNT+WB) and the new ginseng (G+WB) in 8 healthy subjects.

5.5. Chapter Discussion

In the acute study in diabetes, konjac mannan fibre failed to reduce the post-prandial glucose levels. It was postulated that the lack or delay in viscosity of the meal was the cause of the lack of effect observed. The rheological experiment seemed to confirm these results as the konjac did not increase the viscosity of the Ensure. Similarly, in the ginseng experiment, the added konjac had only a small effect on incremental glucose area, which only reached significance when all the konjac meals were pooled. However in the in vivo konjac experiment, the konjac fibre did show a significant, 29-32% decrease in incremental glucose areas. These reductions, although substantial, are still less than the 40-50% reported in the literature (Vuksan et al., 1992). These experiments therefore show that Ensure seems to inhibit gel formation of konjac fibre and this reduces the glucose lowering potential of the fibre in vivo. This effect might be less pronounced in a healthy population. One of the reasons for this might be that in the diabetic population even a mild sub-clinical degree of autonomic neuropathy is reflected in slower gastric emptying which might negate the effect of the viscous fibre as the slower gastric emptying will in turn reduce the rate of glucose absorption in the small intestine (Levitt et al., 1980).
The ginseng experiment again showed that the ginseng used in the acute study had no effect on the glucose tolerance of healthy subjects. However the CNT 2000 ginseng, which had previously shown to be effective, also showed no significant reduction in AUC. A lack of power might be the reason for not finding a significant effect of any of the ginsengs tested, however insufficient supplies of CNT2000 prohibited inclusion of more subjects.

These data therefore suggested that, prior to the start of the long-term trial, a proven, effective ginseng preparation needed to be selected and that the vehicle of konjac administration was of great importance and needed to be investigated further. The next chapter describes the selection of the most effective ginseng from 5 different samples and explores different, effective and palatable means of incorporating konjac fibre into food.
Chapter 6

SELECTION OF EFFECTIVE GINSENG AND METHOD OF KONJAC ADMINISTRATION

6.1. Chapter Synopsis

The experiments performed in Chapter 5 made it clear that, prior to the start of the long-term study, an effective ginseng preparation was urgently needed. In addition the effective type, dose and mode of administration of konjac mannan fibre also needed to be established.

A. Ginseng

As the company, which had in the past supplied us with ginseng, was not in a position to supply new material, ginseng farmers were contacted and 5 batches of different American ginsengs were set aside for in vivo testing and analysis. The ginseng which lowered the postprandial glycaemia to the greatest extent was to be used in further studies.

B. Konjac mannan

To determine the most effective mode and dose of konjac three experiments were undertaken using the standard glycaemic testing protocol. The most viscous form of soluble fibre was used which was a mixture of konjac and xanthan fibre (Viscous Fibre Blend, VFB):

1. To establish most effective mode of incorporation, VFB was incorporated into capsules, bread, or margarine.

2. To establish the minimum effective dose, 1, 2, and 4g of VFB were tested. The VFB was baked into bread or mixed into margarine
3. As time of ingestion of VFB capsules might influence its effect on postprandial glycaemia, in this experiment the capsules were given either at 30min before, immediately before, during or after the meal was consumed.

6.1.1. Results

All five ginsengs tended to lower postprandial glycaemia, the American ginseng from the Broda farm was selected for future studies as it lowered the AUC to the greatest extent.

Incorporation of VFB into capsules and bread had no significant effect on postprandial glycaemia, however mixing of the VFB into margarine reduced the area under the curve by 34% (p<0.001). A repeat of this experiment, but using different levels of the VFB, again showed that incorporation of the VFB into margarine was the most effective in lowering postprandial glycaemia. All doses of VFB when incorporated into margarine reduced the mean postprandial glucose AUC but this only reached statistical significance with the 4g dose. Ingestion of VFB capsules at different times had no effect on postprandial glycaemia.

6.1.2. Conclusion

It was unexpected that the VFB, when incorporated into bread, had no effect, the experiment was therefore repeated and at the same time a dose response was established. This experiment again showed that incorporation of the VFB into margarine was more effective than baking it into bread.

In preparation for the long-term study, the selected ginseng and its control (cornstarch) were sent off to be encapsulated.

A decision needed to be made on how to administer the VFB. When baked into bread it did not seem to be effective. When mixed with margarine it did seem to be effective and a margarine spread could easily be prepared however its limiting factor would be acceptability and the limited amount of VFB which could be incorporated into the margarine. It was therefore decided to use a VFB sprinkle in addition to the margarine spread. Unlike other fibres, sprinkling of VFB on to carbohydrate foods had been shown to be effective previously (personal communication, Dr Vuksan). In
addition, it was decided to also use VFB capsules as some studies had shown that, in the long-term, capsules might be effective in lowering lipid levels (Khan et al., 1981)
6.2. Introduction

The company which had provided us in the past with ginseng capsules, was no longer able to do so. As this was quite unexpected, a reliable, effective preparation of ginseng was therefore urgently needed for my long-term study and other studies planned at the Risk Factor Centre. Although a major part of the work was performed by Dr John Sievenpiper, who was at that time a PhD student with Dr Vuksan, because of the urgency, the selection process was a group effort of Dr Vuksan’s students and staff. Accordingly, in cooperation with the Ontario Ginseng Growers Association, 5 ginseng farms in Simcoe County were contacted. The owners agreed to set aside one batch of 180kg of whole ginseng root each, which allowed us to select the most effective ginseng through in vivo testing and be assured that sufficient quantity from the same batch would be available for future studies.

In preparation for the long-term study, a decision needed to be made on which fibre to use and how to best incorporate it into the diet. At that time one of Dr Vuksan’s students was using a viscous fibre blend, consisting of 75% konjac mannan and 25% xanthan. Using a combination of konjac mannan fibre and xanthan produces a fibre with a much higher viscosity (Table 6.1). In vivo studies confirmed the effectiveness of using the konjac and xanthan viscous fibre blend (VFB) (Vuksan, Unpublished data). To determine the most effective and palatable way of incorporating fibre into the diet, in vivo studies were undertaken in which fibre was administered either in capsules, baked into bread or mixed into margarine.
Table 6.1. Viscosity measurements of three different konjac mannan fibres alone or blended with xanthan gum at two different concentrations. Measurements were taken 120 min after mixing with water (Vuksan, unpublished data)

<table>
<thead>
<tr>
<th></th>
<th>Viscosity at Different Levels of Xanthan (cps)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0% Xanthan</td>
</tr>
<tr>
<td>KJM-1</td>
<td>5,850</td>
</tr>
<tr>
<td>KJM-2</td>
<td>6,630</td>
</tr>
<tr>
<td>KJM-3</td>
<td>8,580</td>
</tr>
</tbody>
</table>

*KJM=konjac-mannan fibre; + viscosity (cps) was calculated by multiplying the dial reading by the factor (390) corresponding to the viscometer spindle (E) and speed (12rpm) combination utilised (Brookfield Hellipath Stand Spindle Factors, Stoughton MA, USA)

6.2.1 Subjective assessment of appetite

It has been suggested that fibre may play a role in weight maintenance through increasing satiety levels. Several studies using guar gum have shown an increase in satiety levels (Kovacs et al., 2001; Kovacs et al., 2002; Pasman et al., 1997) following guar administration but not all (Ellis et al., 1981; Heini et al., 1998). Discrepancies between these results might be because of the different dosages used, study design and the difficulty in quantifying satiety.

As konjac mannan is a viscous fibre it might also be able to influence appetite and satiety (desire to eat, feeling of fullness etc). In previous studies, as part of the standard glycaemic response protocol, subjects were asked to rate their satiety using a bi-polar scale ranging from −3 (extremely hungry) to +3 (uncomfortably full). However interval scales are more powerful instruments because the interval or distance between points on the scale is assumed to be equal (Hetherington and Rolls, 1987). Therefore parametric tests can be applied to the data. The visual analogue scale (VAS) is such a scale. The VAS is a straight line usually measuring 100mm with bipolar anchors of adjectives or descriptive phrases. One end of the scale represents the lowest rating of the variable and the other end the highest rating, e.g. “not at all hungry” and “extremely hungry”. VAS are easy to administer and sensitive to small
changes (Maxwell, 1978). It was therefore decided to replace the old scale and use the VAS instead in future experiments. VAS was used in VFB study 2 and 3.

6.3.1. Selection of Ginseng

Five ginsengs were collected from a range of farms (Broda, Shaw, Duca, Spreit and John Farm) to ensure that varying growing conditions (location, soil type, age at harvest, etc) and physical characteristics such as body shape (short, long, thin, etc.) and the ratio of rootlets to body were included. These differences were verified using a standard questionnaire. The ratio of rootlets-to-body was determined by hand sorting with a 1cm gauge mesh. Expressed as % rootlets, it was 14% for Duca farm, 19% for Shaw Farm, 22% for Broda Farm, 24% for Spreit Farm, and 24% for John Farm. Representative (by rootlets-to-body ratio) 11.5kg samples were taken from each of the five 180kg batches for grinding, ginsenoside analyses, and subsequent clinical screening. The remainder was stored in a central location awaiting the screening outcome. We found that the 5 batches were distinct yet still authentic AG by their ginsenoside profile (Table 6.1).

<table>
<thead>
<tr>
<th>Farm</th>
<th>Protopanaxadiols</th>
<th>Protopanaxatriols</th>
<th>Total</th>
<th>Ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rb1</td>
<td>Rb2</td>
<td>Re</td>
<td>Ref</td>
</tr>
<tr>
<td>Shaw</td>
<td>3.245</td>
<td>0.040</td>
<td>0.238</td>
<td>0.241</td>
</tr>
<tr>
<td>Duca</td>
<td>4.149</td>
<td>0.037</td>
<td>0.224</td>
<td>0.269</td>
</tr>
<tr>
<td>John</td>
<td>4.191</td>
<td>0.039</td>
<td>0.252</td>
<td>0.358</td>
</tr>
<tr>
<td>Broda</td>
<td>4.087</td>
<td>0.056</td>
<td>0.334</td>
<td>0.252</td>
</tr>
<tr>
<td>Spreit</td>
<td>3.294</td>
<td>0.047</td>
<td>0.286</td>
<td>0.317</td>
</tr>
</tbody>
</table>

Applying the same 75g-OGTT protocol as had been used previously and using 9g doses of the 5 AG batches, 2-way ANOVA showed a significant effect of time (P<0.0001) but not treatment (P=0.098) with significant interaction (P=0.026) on plasma glucose. Exploring the interaction with 1-way ANOVAs, it was found that there was a significant effect of treatment at 60min (P=0.015) and on AUC (P=0.046). Comparisons with control showed that all the ginsengs reduced postprandial glucose levels but this only reached significance with the ginsengs from the Broda, John and Spreit Farms (Figure 6.1). Insulin was not measured. Broda Farm ginseng which reduced the AUC to the greatest extent was concluded to have the greatest lowering
effect compared with control. In view of these results, the whole 180kg batch of Broda Ginseng was reserved for the proposed studies.

![Figure 6.1 — Differential effects of American ginseng (AG) batches from 5 different Ontario farms: The line plots and bars represent the incremental change and AUC for plasma glucose and insulin following control (■) or 9g AG (○) taken 40min before a 75g-OGTT in 12 healthy subjects (sex: 5m:7f, age: 27±2y, BMI: 24±1kg/m²). P values are for repeated measures ANOVA with non-orthogonal contrasts comparing treatment with placebo.](image)

6.4. Effective and Palatable Incorporation of VFB

During the long-term study subjects would be required to take 7g of viscous fibre a day for a three month period. To ensure compliance it was therefore crucial that palatable and effective viscous fibre products would be available. In order to be effective in lowering postprandial glycaemia, the viscous fibre needs to be intimately mixed with the food (Jenkins et al., 1979). However preparation of palatable formulations is a challenge.
To investigate the most effective and palatable mode of VFB incorporation three studies were undertaken:

**VFB study 1: Different methods of VFB incorporation:** It was decided that three different methods of VFB incorporation would be tested. Traditionally, incorporation of viscous fibre has been with the carbohydrate portion of the food to ensure adequate mixing. Therefore the first product to be tested was a white bread with added VFB. The disadvantage of white bread is that it limits the choice of carbohydrate source, a spread containing viscous fibre would have the advantage that it could potentially be added to any carbohydrate portion. The second product formulated therefore was a margarine with added fibre. The third product to be tested was VFB in capsules.

**VFB study 2: Dose response:** To establish a minimum effective dose of VFB, a dose response study with 1, 2 and 4g of VFB was undertaken.

**VFB study 3: Timing of capsules:** The possibility that the time of ingestion of VFB capsules would influence its effect on postprandial glycaemia was explored.

### 6.4.1 Protocol

**VFB study 1:** Eight healthy subjects were recruited from the Risk Factor Modification Centre, 3 men and 5 females, (Mean±SD), age 28±7y, BMI 22±4 kg/m². Control and VFB breads were prepared by the Kensington Bakery (Toronto, ON). Test meals consisted of white bread, margarine and capsules with VFB either added to the bread, margarine or capsules with control capsules containing cornstarch (see Table 6.3). Capsules were taken immediately before the white bread test meal. Palatability data were collected using a scale from 1 to 10 where 1 was "extremely dislike" and 10 “delicious".
Table 6.3: VFB study 1: Test meals consumed for the study indicating where the VFB is incorporated.

<table>
<thead>
<tr>
<th>Test Meal</th>
<th>Control</th>
<th>VFB-Marg</th>
<th>VFB-Bread</th>
<th>VFB-Caps</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread</td>
<td>50g CHO (111g)</td>
<td>50g CHO (111g)</td>
<td>50g CHO (147g)</td>
<td>50g CHO (111g)</td>
</tr>
<tr>
<td>Margarine</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
</tr>
<tr>
<td>Capsules (8)</td>
<td>4g cornstarch</td>
<td>4g cornstarch</td>
<td>4g cornstarch</td>
<td>4g VFB</td>
</tr>
<tr>
<td>VFB</td>
<td>0g</td>
<td>4g</td>
<td>4g</td>
<td>4g</td>
</tr>
<tr>
<td>Incorporation of VFB</td>
<td>None</td>
<td>In margarine</td>
<td>In bread</td>
<td>In capsules</td>
</tr>
</tbody>
</table>

VFB - viscous fibre blend

VFB study 2: Twelve healthy subjects 4M, 8F (Mean±SD) BMI 27±4 kg/m², age 36±13y, consumed white bread and margarine (50g of carbohydrate, 10g of margarine) on 8 different occasions. Six test meals had either 1, 2, or 4g of viscous fibre either mixed into the bread or into the margarine; the other 2 test meals had no fibre added and acted as control (Table 6.4).

Table 6.4: VFB study 2: Composition of the eight different test meals which were consumed, including two controls, indicating amount of viscous fibre blend (VFB) in each test meal and whether it was incorporated into the bread or margarine.

<table>
<thead>
<tr>
<th>Test Meal</th>
<th>Control (x2)</th>
<th>Bread-1g</th>
<th>Bread-2g</th>
<th>Bread-4g</th>
<th>Marg-1g</th>
<th>Marg-2g</th>
<th>Marg-4g</th>
</tr>
</thead>
<tbody>
<tr>
<td>White bread</td>
<td>50g CHO</td>
<td>50g CHO</td>
<td>50g CHO</td>
<td>50g CHO</td>
<td>50g CHO</td>
<td>50g CHO</td>
<td>50g CHO</td>
</tr>
<tr>
<td>Margarine</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
<td>10 g</td>
</tr>
<tr>
<td>VFB</td>
<td>0g</td>
<td>1g</td>
<td>2g</td>
<td>4g</td>
<td>1g</td>
<td>2g</td>
<td>4g</td>
</tr>
<tr>
<td>Incorporation of VFB</td>
<td>Into bread</td>
<td>Into bread</td>
<td>Into bread</td>
<td>Into Margarine</td>
<td>Into Margarine</td>
<td>Into Margarine</td>
<td></td>
</tr>
</tbody>
</table>

VFB - viscous fibre blend
**VFB study 3:** The aim of this study was to explore whether the postprandial glucose response to a meal would differ if VFB capsules were taken at different times with respect to a meal, i.e. some time before a meal, immediately before a meal, during the meal or after the meal was consumed. Therefore thirteen healthy subjects 5M, 8F (Mean±SD), BMI 25±5 kg/m², age 39±17y, consumed 50g of carbohydrate from white bread and 18 capsules on 6 different occasions. Each capsule contained either 500mg of cornstarch or 500mg of VFB, the 18 capsules were divided as follows: six capsules were given 30min before the white bread, six capsules immediately before the white bread, and six capsules 15min after white bread ingestion with the exception of one meal where the six capsules were consumed together with the white bread. Two test meals acted as control and all 18 capsules contained only cornstarch. During the other tests meals, 6 VFB capsules (3g of VFB) were substituted for 6 cornstarch capsules and were taken at either -30min (VFB at -30), immediately before (VFB at 0), during the meal (VFB with meal) or 15 min after the white bread was eaten (VFB at 15)(see figure 6.2 and table 6.5).

![Fig 6.2. Schematic representation of study protocol used in the VFB study 3 – Timing of Capsules. Fingerprick blood samples were taken at all time points. Capsules were taken at -30min, 0min and 15min with 200ml of water. White bread was consumed after the 0min blood sample.](image-url)
Table 6.5.: Table illustrating the content of the 6 capsules given at each time point during the different test meals. Each capsules contains either 500mg of cornstarch (CS) or viscous fibre blend (VFB).

<table>
<thead>
<tr>
<th>Content of Capsules at Different Time Points</th>
<th>0min</th>
<th>15min</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 control meals:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>CS</td>
<td>CS</td>
</tr>
<tr>
<td>Control</td>
<td>CS</td>
<td>CS</td>
</tr>
<tr>
<td>4 test meals:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VFB at -30</td>
<td>VFB</td>
<td>CS</td>
</tr>
<tr>
<td>VFB at 0</td>
<td>CS</td>
<td>VFB (before meal)</td>
</tr>
<tr>
<td>VFB with meal</td>
<td>CS</td>
<td>VFB (with meal)</td>
</tr>
<tr>
<td>VFB at 15</td>
<td>CS</td>
<td>CS</td>
</tr>
</tbody>
</table>

CS-cornstarch, VFB-viscous fibre blend

The standard finger prick protocol was used for all three studies (see chapter 2). For VFB studies 2 and 3, a visual analogue scale (VAS) was used to record satiety and replaced the bi-polar scale used in previous studies.

6.5. Results

VFB study 1: Different methods of VFB incorporation: All meals were well tolerated. There were no significant differences in starting levels (Mean±SEM)(Control 4.4 ± 0.2; VFB-bread 4.6 ± 0.1; VFB-marg 4.5 ± 0.1; VFB-caps 4.7 ± 0.1mmol/L, p<0.3). Ad hoc analysis showed a significantly decreased postprandial
glucose level at 30min after the meal which had VFB mixed into the margarine (p<0.0006)(Fig 6.2), similarly the area under the curve was significantly decreased after the VFB-margarine meal compared to the other meals (p<0.03) (Fig 6.3).

Fig 6.3. Postprandial glucose of 8 healthy subjects levels after consuming white bread, margarine and capsules containing 50g of carbohydrate and 8g of fat. Four grams of VFB was added either to the bread (VFB-bread), margarine (VFB-marg) or capsules (VFB-caps). Control capsules contained 4g cornstarch. Data are expressed as mean±SEM. P values are for repeated measures ANOVA comparing treatment with control. **p<0.0006.
Fig 6.4. Postprandial glucose areas of 8 healthy subjects after consuming white bread, margarine and capsules containing 50g of carbohydrate and 8g of fat. Four grams of VFB was added either to the bread (VFB-bread), margarine (VFB-marg) or capsules (VFB-caps). Control capsules contained 4g cornstarch. Data are expressed as mean±SEM. Repeated measures ANOVA was used to compare treatment with control, bars with different letters are significantly different (p<0.03).

Palatability of the VFB-margarine meal was significantly lower than the other VFB containing meals but did not differ from the control meal (p<0.05) (Fig 6.3). Satiety scores, using the bi-polar scale, showed no significant differences between the test meals at any time point.

Fig 6.5: Palatability scores 8 healthy subjects after consuming white bread, margarine and capsules containing 50g of carbohydrate and 8g of fat. Four grams of VFB was added either to the bread (VFB-bread), margarine (VFB-marg) or capsules (VFB-caps). Control capsules contained 4g cornstarch. Data are expressed as mean±SEM. Repeated measures ANOVA was used to compare treatments with control, bars with different letters are significantly different (p<0.05).
**VFB study 2: Dose Response**: Two factor repeated measures ANOVA over the time course of the experiment showed a significant difference in glucose concentrations between treatments using the Newman-Keuls multiple-comparison test a significant difference between control and Marg-4g was found. Post-hoc analysis showed that there were no significant differences in starting values (Table 6.4), in addition there were no differences in postprandial response between control and Bread-1g, Bread-2g and Bread-4g. However when VFB was mixed with margarine the postprandial glycaemia was significantly reduced at 30 and 45min for all three doses (p<0.001)(Fig 6.5). Compared to control, VFB containing meals (Bread-1g, Bread-2g, Bread-4g, Marg-1g, Marg-2g, and Mar-4g) reduced the incremental AUC by 2%, 5%, 20%, 22%, 24%and 34% respectively, but this only reached statistical significance for the Marg-4g test meal (p<0.001)(Fig 6.6).

**Table 6.6**: Fasting capillary blood glucose values for each test meal consumed during the dose response-VFB study 2.

<table>
<thead>
<tr>
<th>Testmeal</th>
<th>Control (x2)</th>
<th>Bread-1g</th>
<th>Bread-2g</th>
<th>Bread-4g</th>
<th>Marg-1g</th>
<th>Marg-2g</th>
<th>Marg-4g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>4.5±0.1</td>
<td>4.4±0.1</td>
<td>4.4±0.2</td>
<td>4.5±0.1</td>
<td>4.6±0.1</td>
<td>4.5±0.1</td>
<td>4.5±0.1</td>
</tr>
</tbody>
</table>
Fig 6.6: Postprandial glucose of 12 healthy subjects levels after consuming white bread, and margarine containing 50g of carbohydrate and 8g of fat. One, 2 or 4g of VFB was added either to the bread (Bread-1g, Bread-2g, Bread-4g) or margarine (Marg-1g, Marg-2g, Marg-4g). Control is the mean of two control test meals. Data are expressed as mean±SEM. *significantly different from control p<0.001.

Fig 6.7: Incremental glucose AUC of 12 healthy subjects levels after consuming 50g of carbohydrate from white bread, and 8g of fat from margarine. One, 2 or 4g of viscous fibre blend (VFB) was added either to the bread (Bread-1g, Bread-2g, Bread-4g) or margarine (Marg-1g, Marg-2g, Marg-4g). Control is the mean of two control test meals. Data are expressed as mean±SEM. Bars with different letter are statistically different, p< 0.002.
Using the VAS, there were no significant differences seen in either hunger, desire to eat, fullness or thirst at any time between the meals. Palatability scores were 8.9±0.3, 8.4±0.4, 8.7±0.4, 8.9±0.4, 8.1±0.6, 7.3±0.8, 7.0±0.9 for Control, Bread-1g, Bread-2g, Bread-4g, Marg-1g, Marg-2g, and Marg-4g respectively and although the palatability score tended to be lower for the meals which had VFB incorporated into the margarine this did not reach significance using the New-Keuls multiple comparison test (p=0.04).

VFB study 3: Timing of Capsules: Two factor repeated measures ANOVA over the time course of the experiment showed no significant glucose and treatment interaction. There were no significant differences in AUC (Fig 6.8), palatability score and VAS.

![Timing of VFB Capsules](image)

Fig 6.8. Incremental glucose AUC of 12 healthy subjects levels after consuming 50g of carbohydrate from white bread and 18 capsules. Six capsules containing cornstarch or VFB were consumed either 30min before, immediately before, during or 15min after bread consumption. Control is the mean of two control test meals. Data are expressed as mean±SEM.
6.6 Chapter Discussion

Due to the concerted efforts of Dr Vuksan’s team we were able to select a ginseng which significantly lowered the postprandial glycaemia. As a consequence of these results the Broda batch was ground and encapsulated in readiness for the long-term study.

The three konjac/VFB studies confirmed that method of incorporation is an important determinant of the ability of viscous fibre to lower postprandial glycaemia. The finding that the viscous fibre was more effective when mixed with the margarine was unexpected but was again confirmed in the second VFB study. Previous studies have demonstrated that just sprinkling the viscous fibre on the meal negates its glucose lowering effect (Williams et al., 1980), however the margarine, presumably because it is spread over the carbohydrate portion of the meal, allows the viscous fibre to be intimately mixed with the carbohydrate. Previous effective konjac preparations used biscuits and crackers (Vuksan et al., 1999b), it is interesting that the higher fat cookies tended to be more effective than the lower fat crackers. However, the reason that the viscous fibre/margarine method was more effective than baking it in the bread is still unclear. One can speculate that the usual separation of the fat and liquid phases in the stomach did not take place when the VFB was mixed with the margarine. This dispersion may have caused a slowing of gastric emptying and therefore reduced the rate of glucose absorption in the small intestine.

It was unfortunate that the most effective meals, i.e. the 4g margarine/VFB meals received the lowest palatability scores, again emphasising the difficulty in preparing palatable, effective VFB preparations.

In view of these results it was decided that the VFB would be offered to the subjects in three different preparations: a margarine spread, a sprinkle and in capsules. The drawback of the margarine spread is that not all subjects use margarine routinely and introducing it into their diet would alter their nutrient profile. Furthermore, in order to ingest 7g of VFB, subjects would be required to ingest approximately 20g of margarine which would be more than people habitually consume. It was therefore
decided to also offer a sprinkle, the reason a VFB sprinkle was selected in that it allowed it to be added to all meals and snacks. In addition, contrary to other viscous fibres, konjac has been reported to be effective when sprinkled on the carbohydrate part of the meal (personal communication, Dr Vuksan).

Capsules are an ideal method of viscous fibre administration as they are highly acceptable and therefore compliance rates are high, especially in a diabetic population habituated to taking medications on a regular basis. Although viscous fibre capsules do not have an acute beneficial effect on blood glucose possible long-term beneficial effects may be present, especially on lipid levels. When 9g/day of guar gum was administered in capsules to 24 healthy volunteers for four weeks, significant decreases in Total, and LDL-cholesterol were observed (Khan et al., 1981). Capsules might therefore have beneficial long-term effects which may not be predicted from acute studies. It was estimated that the total amount of VFB to be ingested which would be acceptable to subjects using the sprinkle and margarine spread, would be approximately 3-4g/day. Previous studies (Vuksan et al., 1999a; Vuksan et al., 1999b) used doses of konjac ranging from 6-13g. Therefore, to increase the total viscous fibre dose, it was decided that subjects would also be given VFB capsules.
Chapter 7
LONG-TERM STUDY

7.1 Chapter Synopsis

Both American ginseng and viscous fibre have been shown to improve diabetes control, American ginseng by increasing post-prandial insulin levels and viscous fibre by improving insulin sensitivity.

7.1.1 Objectives

To investigate the combined effects of American Ginseng (AG) and a highly viscous fibre blend (VFB) on metabolic control in type 2 diabetes.

7.1.2 Methods

A partially blinded, randomised, placebo controlled, crossover design was used with a 4 week run-in period, two 12 week treatment phases separated by a 4 week wash-out period. Thirty subjects with well-controlled type 2 diabetes, participated in the study (18M, 12F; age 64±7y; BMI 28±5kg/m²; HbA1c 7.0±1.0%). Twenty-three subjects were treated with oral hypoglycaemic agents and 7 with diet alone. Medications, dietary intake, and lifestyle were kept constant throughout. During the control phase subjects were given 3g of cornstarch (placebo for AG) together with 14g wheat bran (fibre matched placebo for VFB) and during the test phase 3g of AG together with 7g of VFB.

7.1.3 Results

Compliance was generally good, percentages of the prescribed supplements consumed during the Control and Test periods were as follows: cornstarch 90% (2.7±0.08 g), ginseng 90% (2.7±0.07 g), wheat bran 73% (10.3±0.7 g), VFB 91% (6.4±0.3 g). Using the GLM repeated measures ANOVA significant differences between
treatments were found for HbA1c (p<0.02), Total cholesterol (p<0.03), LDL-cholesterol (p<0.04), and Apolipoprotein B (p<0.02). There were no significant differences between the treatments in fasting glucose and insulin, Quicki, HOMA, triacylglycerol, HDL cholesterol, Apolipoprotein A-1, C-reactive protein, oxidized LDL, and blood pressure measurements. The percent difference at 12 week from control for HbA1c was (-4.1±1.4%, p<0.01), total cholesterol (-5.7±1.9%, p<0.01), LDL cholesterol (-8.2±3.1%, p<0.002, and apolipoprotein B (-9.0±2.3%, p<0.0005).

Both the total amount of the combined supplement and the VFB dose by itself correlated negatively with the difference between changes in control and test HbA1c levels (r=-0.27, n=30, p<0.04).

7.1.4 Conclusion

Addition of American ginseng and viscous fibre to conventional treatment significantly decreases HbA1c levels and improves cardiovascular risk factors over a 12 week trial in well-controlled individuals with type 2 diabetes.
7.2 Introduction

The aim of the study was to assess the long-term metabolic and therapeutic effects of combining a viscous fibre blend (VFB) and American Ginseng (*Panax quinquefolius*) in the management of type 2 diabetes, the primary and secondary objectives were:

- **Primary**: To explore the effects of using the combination therapy ginseng and konjac mannan for 3 months on HbA1c in type 2 diabetes.
- **Secondary**: To evaluate the effect of the treatment on, fasting blood glucose, fasting insulin, blood pressure, insulin sensitivity, total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglycerol, oxidised LDL, apolipoproteins A-1 and B, and C-reactive protein.

7.3 Research Design And Methods

7.3.1. Subjects

7.3.1.1. Power Calculation

Given that the study employed a crossover design, we wanted to be able to detect a 0.8% treatment difference in HbA1c (SD=1.3%). Power analysis calculations indicated that approximately 28 individuals were required ($\alpha=0.05$ and $\beta=0.8$), assuming a 30% attrition rate a total of 37 subjects were to be recruited. The standard deviations and attrition rate used were based on our own previous observations and those of other investigators from studies performed in type 2 diabetes.

7.3.1.2. Recruitment

The study was approved by the St Michael’s Hospital Ethics board, and informed consent was obtained from all subject prior to the start of the study. Subjects were recruited through newspaper advertisements and from patients already attending the diabetes clinic at the Risk Factor Modification Centre at St. Michael’s Hospital.
Inclusion criteria were: presence of type 2 diabetes (at least 1 year) as defined by HbA₁c between ≥6.5% and ≤8.4% (140% of normal) at recruitment; being treated with diet or oral hypoglycaemic medication; be between the ages of 40 and 75 years; have systolic blood pressure < 140 and diastolic blood pressure < 90; be clinically euthyroid; have normal renal and liver functions; live within 30 km range from St. Michael’s Hospital; and be willing to comply with the study protocol and sign a consent form.

Interested volunteers were initially screened by telephone using a simple questionnaire and invited to come to the Centre where the exact nature of the study was described and volunteers had the opportunity to ask detailed questions about the study. If written consent was obtained, potential candidates filled-out a detailed questionnaire concerning their medical history, drugs and medication use (including ginseng, herbs, vitamin and minerals), smoking habits, alcohol intake and exercise pattern, and whether they were already following a specific diet. This visit was followed by a screening visit for which the subjects were asked to come to the clinic after a 10-12-hour overnight fast. A blood sample was taken and anthropometric and clinical blood pressure measurements were collected. Blood was analysed by the St Michael’s Hospital laboratory using standard techniques (see Chapter 2) for total cholesterol, LDL-cholesterol (calculated), HDL-cholesterol and triacylglycerol, as well as for HbA₁C and FBG to confirm diagnosis, level of diabetes control and eligibility for the study. Those volunteers who were willing to participate in the study and met inclusion/exclusion criteria were invited to join the study.

Fifty-two subjects were screened and of those, 39 were recruited. During the study period 9 subjects dropped out (23%). Subjects #11 and 18 because of a change in their medication, #27 because of gastrointestinal side effects, #22 felt unable to continue taking the capsules, #15, 20, 23, 25, and 31 felt they were unable to continue the study due to lack of time. Therefore a total of 30 subjects (18 men and 12 women) completed the study with a mean age (mean±SD) of 64±7 y, BMI of 28±5kg/m², HbA₁c 7.0±1.0%, duration of diabetes of 9.0±6.8y. Seven were treated with diet alone, 23 with oral hypoglycaemic agents.
During the screening visit, subjects were instructed on details of the study protocol such as completing the dietary record, VAS and other questionnaires. They were asked to maintain a consistent level of physical activity, and to continue their habitual diet and lifestyle during the course of the study. Every effort was made through counselling to ensure subjects did not change body weight. Also, medications for the control of diabetes, if taken, were kept unchanged starting at least 3 months prior to the beginning of the study.

7.3.2. Study Protocol

7.3.2.1. Design

The study used a partially blinded, randomised, placebo controlled, crossover design (see Fig 7.1). Due to the nature of the fibre it was impossible to blind the subjects to which fibre they were on. (see section 7.3.2.3. describing the supplements). The study was divided into two phases with each phase having a 4 week run-in period, and a 12 week treatment period, separated by a 4 week wash-out period.

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Fig 7.1 Schematic representation of the study protocol used in the long-term study.

The treatments consisted of an *ad-libitum* Canadian Diabetes Association recommended diet supplemented with either cornstarch capsules and wheat bran...
(Control) or American ginseng capsules and VFB (test). Subjects were asked to attend the clinic at weeks -4, 0, 3, 6, and 12 during each phase. Table 7.1 summarises the analysis and measurements performed at each visit, and section 7.3.2.3. describes the methods of collection.

Table 7.1: Analysis and measurements collected at each clinic visit during the study.

<table>
<thead>
<tr>
<th>Visit (weeks)</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screen-run</td>
<td>-4</td>
<td>Run-in</td>
</tr>
<tr>
<td>Blood sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA1c</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Lipids</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>FBG</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Oxidised LDL</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Liver &amp; kidney function</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Anthropometric measurements</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Clinical Blood pressure</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3 day Diet record</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Quality of Life Questionnaire</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>OGTT</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

OGTT - oral glucose tolerance

7.3.2.2. Study details and measurements:

On weeks -4, 0, 3, 6 and 12 of each treatment phase, subjects attended the clinic at the Risk Factor Modification Centre (RFMC), at St. Michael’s Hospital after a 10 - 12 hour overnight fast. The only exception was on week 3 when subjects did not have to come fasting as no blood work was taken that day; this visit was used to
review with the subjects their supplement intake and discuss any problems they had encountered. To measure compliance, capsules and fibre supplements not consumed were returned and capsule number and weight of the spread and sprinkle were recorded. Blood samples were drawn at each visit except for visit 3 and were analysed for HbA1c, Total, LDL- and HDL-cholesterol, triacylglycerol, NEFA, Apolipoproteins A and B, fasting blood glucose, fasting insulin, insulin sensitivity, C-reactive protein, and oxidised LDL. Blood samples were drawn by trained intravenous nurses and analysed according to established methodologies (see Chapter 2).

Anthropometric measurements included body weight and body fat composition using infra red technology (Futrex 5000). Clinical blood pressure was measured at each visit using a digital blood pressure monitor (OMRON, HEM-907, Vernon Hills, USA). Three-day food records were collected and weeks 0, 6 and 12 of each treatment arm were analysed for macronutrient and fibre content using the Food Processor for Windows diet analysis program (ESHA Research, version 6.11, Salem, USA). A 75g OGTT was performed at the beginning and end of each phase. This would allow an estimation of insulin sensitivity using the formula proposed by Matsuda et al (Matsuda and DeFronzo, 1999). A symptom questionnaire was completed at each visit. Electrolytes, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), prothrombin time (PT), international normalised ratio (INR) and activated partial thromboplastin time (APTT) performed to monitor health status of volunteers and analysed in the main hospital laboratory (see table 7.1).

7.3.2.3. Supplements:

GINSENG: The ginseng supplement consisted of 3g of ginseng or 3g of cornstarch which acted as the placebo. Each supplement was contained in 6 gelatin capsules (500mg/capsule). There were no differences in macro-nutrient composition between the capsules (Table 7.2). The subjects were instructed to take two capsules before each meal (Fig 7.3). The ginseng used came from the Broda Farm (see Chapter 6) and had been donated by the Ontario Ginseng Growers. Placebo capsules, on the other hand, contained cornstarch (International Nutrition Ltd, Markham, Ontario).
There were no visually detectable differences between the placebo and ginseng capsules.

**FIBRE:** The aim was to supplement the diet with 6g of fibre, therefore 7g of VFB and 14g of wheat bran needed to be consumed. Subjects were instructed to consume seven grams of VFB per day in the form of capsules (4g), margarine and/or powder. The 14g of white wheat bran control supplement (Grain Process Enterprises Ltd, Scarborough, Ontario) was supplied in the same manner as the VFB. Subjects were instructed to take 2 capsules containing the fibre before each meal with a glass of water to ensure that these were swallowed easily. If subjects habitually consumed margarine they were offered the spread and instructed to substitute this for their regular margarine. One serving (1 tsp of KJM margarine or 1.5 tsp of wheat bran margarine) was to be consumed with the meal. If no margarine was taken with a meal, subjects were asked to sprinkle one spoonful of the sprinkle on their food using the supplied spoon. This ensured that at each meal fibre was supplied in two different formats i.e. in a capsule and as a spread/sprinkle. As the volume of the KJM and wheat bran differed, different spoons were supplied on each phase to ensure the correct dose was used (table 7.3). At the beginning of each treatment phase, subjects were advised to introduce the fibre slowly into their diet to avoid gastrointestinal symptoms and increase the dose to the recommended level as tolerated. Although the capsules looked identical, due to the nature of the fibres, blinding the subjects to which fibre they were on was impossible, however they were not told which fibre was expected to have a metabolic effect.

**Table 7.2:** Macronutrient composition of 3g of ginseng and cornstarch supplements.

<table>
<thead>
<tr>
<th></th>
<th>American Ginseng (3g)</th>
<th>Cornstarch (3g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy (kcal/g)</strong></td>
<td>10.5</td>
<td>10.3</td>
</tr>
<tr>
<td><strong>Carbohydrates (g)</strong></td>
<td>2.2</td>
<td>1.7</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>0.21</td>
<td>0.76</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>0.11</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Table 7.3: Number of capsules and amount of ginseng, cornstarch and fibre in the capsules and sprinkle/spread taken at each meal during the treatment periods.

<table>
<thead>
<tr>
<th></th>
<th>Breakfast</th>
<th>Lunch</th>
<th>Dinner</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginseng Capsules</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Konjac Capsules</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Konjac Sprinkle/Spread</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3g</td>
</tr>
<tr>
<td>Cornstarch Capsules</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Wheat bran Capsules</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Wheat bran Sprinkle/Spread</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>10.5g</td>
</tr>
</tbody>
</table>

7.4 Statistical Analysis

Statistical analysis was performed using the SAS software (SAS version 8.2, 2001; SAS Institute). The GLM was used to perform two-way ANOVA to detect differences of outcome variables in treatments and visits, controlling for treatment sequence and sex. If the effect of treatment was significant than percent differences were calculated between weeks 12 of test and control and analysed using the GLM repeated measures one-way ANOVA controlling for sex. Indices of glucose and insulin sensitivity were assessed. As the samples from the OGTT were unusable to analyse insulin (see Chapter 2), insulin sensitivity was calculated using fasting glucose and insulin levels by two different methods: HOMA insulin sensitivity according to the formula: 22.5/FPG×FPI (Matthews et al., 1985) and the modified QUICKI according to the formula 1/log(fasting glucose)+log(fasting insulin)+log(fasting NEFA) (Perseghin et al., 2001). A Pearson regression correlation
was used to analyse the relationship between dose and metabolic response. All results were expressed as mean±SEM and considered statistically significant if p < 0.05.

7.5 Results

7.5.1 Compliance and Anthropometric Measurements

Compliance was generally good, percentages of the prescribed supplements during the Control and Test periods were as follows: cornstarch 90 % (2.7± 0.08 g), ginseng 90% (2.7±0.07 g), wheat bran 73% (10.3±0.7 g), VFB 91% (6.4±0.3 g). Actual daily fibre intake from the supplements during the study was therefore 4.9g from wheat bran and 5.5 g from VFB. There were no changes in body weight, % body fat or waist to hip ratio over the test or control periods (table 7.4).

7.5.2 Background diet

Three day dietary records were analysed for weeks 0, 6 and 12 of each treatment period. No significant changes in macronutrient intake over the treatment periods or between treatments were seen (table 7.4).
Table 7.4: Absolute and percent macronutrient and fibre intake at weeks 0, 6 and 12 during the control and test periods.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 6</td>
</tr>
<tr>
<td><strong>Energy, (kcal/d)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1685±78</td>
<td>1773±120</td>
<td>1776±116</td>
</tr>
<tr>
<td><strong>Protein (g/d)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>86±6</td>
<td>92±7</td>
<td>91±7</td>
</tr>
<tr>
<td>20%</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td><strong>Available Carbohydrate (g/d)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>208±23</td>
<td>217±17</td>
<td>213±16</td>
</tr>
<tr>
<td>49%</td>
<td>49%</td>
<td>48%</td>
</tr>
<tr>
<td><strong>Total Fat (g/d)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60±5</td>
<td>64±6</td>
<td>65±6</td>
</tr>
<tr>
<td>32%</td>
<td>32%</td>
<td>33%</td>
</tr>
<tr>
<td>-SFA</td>
<td>6.2±0.7</td>
<td>6.5±0.9</td>
</tr>
<tr>
<td>-MUFA</td>
<td>21.7±1.6</td>
<td>24.5±3.0</td>
</tr>
<tr>
<td>-PUFA</td>
<td>11.1±0.9</td>
<td>12.1±1.3</td>
</tr>
<tr>
<td><em><em>Dietary Fibre</em> (g/d)</em>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>23±3</td>
<td>22±3</td>
<td>22±2</td>
</tr>
</tbody>
</table>

*Fibre supplied by diet only and does not include the fibre in the supplements.

7.5.3. Outcomes

Using the GLM repeated measures ANOVA over the entire treatment period showed a significant difference in HbA1c (p<0.02), total cholesterol (p<0.03), LDL-cholesterol (p<0.04), and Apolipoprotein B (p<0.02) between treatments. Using the same procedure there were no significant differences between treatments in fasting plasma glucose or insulin levels, HOMA, QUICKI, triacylglycerides, HDL-cholesterol, Total cholesterol:IDL, apolipoprotein A-I, conjugated dienes/LDL, blood pressure, C-reactive protein (table 7.5, and fig 7.3).

Baseline values did not differ significantly between the two treatments (Table 7.5). The percent difference at 12 week from control for HbA1c was (-4.1±1.4%, p<0.01), total cholesterol (-5.7±1.9%, p<0.01), LDL cholesterol (-8.2±3.1%, p<0.002, and apolipoprotein B (-9.0±2.3%, p<0.0005).
Table 7.5: Anthropometric, HbA1c, glucose, insulin, blood lipids, oxidised LDL, c-reactive protein and blood pressure data on weeks 0, 6, and 12 on the control and test periods (mean±SEM).

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th></th>
<th></th>
<th>TEST</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 6</td>
<td>Week 12</td>
<td>Week 0</td>
<td>Week 6</td>
<td>Week 12</td>
</tr>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>80.7±3.2</td>
<td>80.5±3.2</td>
<td>80.8±3.3</td>
<td>80.7±3.2</td>
<td>78.5±3.6</td>
<td>80.9±3.3</td>
</tr>
<tr>
<td><strong>Body fat (%)</strong></td>
<td>28.4±1.2</td>
<td>28.7±1.3</td>
<td>31.2±1.8</td>
<td>28.6±1.2</td>
<td>30.9±2.7</td>
<td>29.5±1.2</td>
</tr>
<tr>
<td><strong>HbA1c (%)</strong></td>
<td>6.94±0.16</td>
<td>6.62±0.17</td>
<td>6.77±0.18</td>
<td>6.89±0.17</td>
<td>6.54±0.15</td>
<td>6.46±0.16***</td>
</tr>
<tr>
<td><strong>Glucose (mmol/L)</strong></td>
<td>7.5±0.4</td>
<td>7.6±0.4</td>
<td>7.5±0.4</td>
<td>6.9±0.4</td>
<td>7.2±0.3</td>
<td>7.3±0.3</td>
</tr>
<tr>
<td><strong>Insulin (pmol/L)</strong></td>
<td>65.3±8.0</td>
<td>63.7±7.3</td>
<td>70.9±6.2</td>
<td>65.8±7.8</td>
<td>63.4±7.7</td>
<td>67.0±7.7</td>
</tr>
<tr>
<td><strong>NEFA (µmol/L)</strong></td>
<td>0.58±0.04</td>
<td>0.60±0.06</td>
<td>0.50±0.03</td>
<td>0.56±0.04</td>
<td>0.55±0.04</td>
<td>0.56±0.04</td>
</tr>
<tr>
<td><strong>HOMA (ratio)</strong></td>
<td>0.070±0.005</td>
<td>0.073±0.010</td>
<td>0.066±0.010</td>
<td>0.081±0.010</td>
<td>0.076±0.010</td>
<td>0.070±0.008</td>
</tr>
<tr>
<td><strong>QUICKI (ratio)</strong></td>
<td>0.44±0.02</td>
<td>0.45±0.02</td>
<td>0.45±0.02</td>
<td>0.47±0.02</td>
<td>0.46±0.02</td>
<td>0.44±0.02</td>
</tr>
<tr>
<td><strong>Cholesterol (mmol/L)</strong></td>
<td>4.64±0.18</td>
<td>4.63±0.19</td>
<td>4.71±0.19</td>
<td>4.62±0.19</td>
<td>4.42±0.16</td>
<td>4.40±0.17**</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>2.66±0.05</td>
<td>2.75±0.14</td>
<td>2.77±0.16</td>
<td>2.77±0.14</td>
<td>2.57±0.13</td>
<td>2.48±0.14*</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td>1.26±0.05</td>
<td>1.21±0.02</td>
<td>1.25±0.04</td>
<td>1.21±0.04</td>
<td>1.22±0.05</td>
<td>1.23±0.04</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td>3.80±0.18</td>
<td>3.89±0.16</td>
<td>3.83±0.14</td>
<td>3.89±0.16</td>
<td>3.74±0.15</td>
<td>3.67±0.14</td>
</tr>
<tr>
<td><strong>Oxidised LDL</strong></td>
<td>2.20±0.13</td>
<td>2.32±0.13</td>
<td>2.24±0.12</td>
<td>2.34±0.13</td>
<td>2.19±0.44</td>
<td>2.07±0.12</td>
</tr>
<tr>
<td><strong>LDL conjugated dienes (µmol)</strong></td>
<td>70.5±8.3</td>
<td>113.3±10.4</td>
<td>61.9±6.7</td>
<td>85.1±11.9</td>
<td>115.6±10.8</td>
<td>58.6±7.4</td>
</tr>
<tr>
<td><strong>LDL conjugated dienes/LDL (µmol/mmol)</strong></td>
<td>26.4±2.8</td>
<td>41.7±3.6</td>
<td>22.9±2.4</td>
<td>29.9±3.3</td>
<td>46.8±4.4</td>
<td>24.8±3.1</td>
</tr>
<tr>
<td><strong>TAG (mmol/L)</strong></td>
<td>1.55±0.14</td>
<td>1.47±0.10</td>
<td>1.53±0.14</td>
<td>1.42±0.10</td>
<td>1.38±0.10</td>
<td>1.53±0.14</td>
</tr>
<tr>
<td><strong>Apolipoproteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ApoA-1 (g/L)</strong></td>
<td>1.55±0.04</td>
<td>1.53±0.04</td>
<td>1.54±0.04</td>
<td>1.51±0.04</td>
<td>1.52±0.04</td>
<td>1.51±0.04</td>
</tr>
<tr>
<td><strong>ApoB (g/L)</strong></td>
<td>0.94±0.04</td>
<td>0.91±0.04</td>
<td>0.98±0.04</td>
<td>0.94±0.04</td>
<td>0.86±0.04</td>
<td>0.88±0.04***</td>
</tr>
<tr>
<td><strong>CRP (mg/L)</strong></td>
<td>2.03±0.36</td>
<td>2.28±0.44</td>
<td>2.44±0.62</td>
<td>2.31±0.43</td>
<td>2.34±0.50</td>
<td>1.93±0.42</td>
</tr>
<tr>
<td><strong>Blood pressure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Systolic (mmHg)</strong></td>
<td>130±3</td>
<td>128±3</td>
<td>133±3</td>
<td>128±4</td>
<td>129±3</td>
<td>130±3</td>
</tr>
<tr>
<td><strong>Diastolic (mmHg)</strong></td>
<td>76±2</td>
<td>73±1</td>
<td>76±2</td>
<td>74±2</td>
<td>73±2</td>
<td>75±2</td>
</tr>
<tr>
<td><strong>Waist (cm)</strong></td>
<td>93.5±2.5</td>
<td>90.7±3.1</td>
<td>93.4±2.6</td>
<td>93.5±2.4</td>
<td>89.6±3.6</td>
<td>91.1±3.6</td>
</tr>
<tr>
<td><strong>Hip (cm)</strong></td>
<td>102.0±1.9</td>
<td>100.2±1.9</td>
<td>101.7±2.0</td>
<td>101.9±2.0</td>
<td>100.8±1.9</td>
<td>101.7±1.8</td>
</tr>
<tr>
<td><strong>Waist:HIP</strong></td>
<td>0.9±0.01</td>
<td>0.9±0.02</td>
<td>0.9±0.01</td>
<td>0.9±0.01</td>
<td>0.89±0.03</td>
<td>0.88±0.03</td>
</tr>
</tbody>
</table>

GLM repeated measures, significant difference between treatments: ***p<0.02, **p<0.03, *p<0.04
7.5.4. Safety Parameters.

Safety factors were measured at the beginning and end of each treatment period to monitor possible changes in kidney and liver function. No statistical or clinical significant changes were observed over the treatment periods or between treatments (table 7.6).

Table 7.6: Safety parameters measured at the beginning and end of the control and test treatment periods.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>TEST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 12</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>137.7±0.4</td>
<td>138.4±0.5</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.4±0.2</td>
<td>4.5±0.1</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>100.3±0.6</td>
<td>101±0.6</td>
</tr>
<tr>
<td>Total CO₂ (mmol/L)</td>
<td>27.1±0.4</td>
<td>27.0±0.6</td>
</tr>
<tr>
<td>Anion Gap (mmol/L)</td>
<td>10.3±0.4</td>
<td>9.9±0.4</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.9±0.3</td>
<td>6.0±0.3</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>79.0±3.0</td>
<td>78.9±4.4</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>25.7±1.7</td>
<td>25.9±1.4</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>28.2±2.7</td>
<td>27.6±2.0</td>
</tr>
<tr>
<td>PT (s)</td>
<td>10.6±0.1</td>
<td>10.0±0.2</td>
</tr>
<tr>
<td>INR</td>
<td>1.0±0.0</td>
<td>1.0±0.0</td>
</tr>
<tr>
<td>APTT (s)</td>
<td>27.5±0.6</td>
<td>27.0±0.6</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanine aminotransferase; PT: prothrombin time; INR: international normalised ratio; APTT: activated partial thromboplastin time. s: seconds
7.5.5 Symptoms

During each visit to the clinic subjects were asked whether they experienced symptoms or conditions such as headaches, flatulence, belching, diarrhoea, nausea, joint pain etc. If their response was positive they were asked to rate the severity on a scale from 1 to 7. Statistical analysis showed no difference between the severity of the symptoms between treatments (fig 7.2).

![Graph showing percent symptom occurrence on weeks 0, 6 and 12 during control and test periods.](image)

Fig 7.2: Percent symptom occurrence on weeks 0, 6 and 12 during control and test periods.
Fig 7.3. Absolute values are given for HbA1c, total cholesterol, LDL-cholesterol, and Apolipoprotein B at weeks 0, 6 and 12 on control and test treatments in 30 patients with type 2 diabetes. Results are mean ± SEM. P values denote significant differences between treatments using GLM ANOVA repeated measures model.
7.5.7. Oral Glucose Tolerance Test

There were no statistically significant differences in plasma glucose values at any time between the test and control groups either at week 0 or week 12 (Fig 7.4). As discussed in Chapter 2, the insulin results for the oral glucose tolerance test could not be analysed.

Fig 7.4: Plasma glucose levels during the oral glucose tolerance performed at week 12 after either the test or the control period. Results are expressed as Mean±SEM.

7.5.8. Dose response

The relationship between total self-reported intake of either ginseng, VFB or both with HbA1c, total cholesterol, LDL-cholesterol and Apo B was also investigated. Only the difference between changes of treatment and control in HbA1c correlated positively with both the total dose (p<0.04) and the VFB (p<0.03) (Fig 7.5).
7.6 Chapter Discussion

In this group of subjects the compliance with the prescribed dosages of ginseng and VFB was very high and the supplements were generally well tolerated. Symptoms associated with high fibre diets such as flatulence, bloating, belching all increased on the VFB arm of the study, but this did not reach significance. The increase in symptoms was especially seen at week 6 but a decrease in symptoms was seen at week 12 as presumably adaptation to the higher fibre intake took place.

The expected difference between treatments in HbA1c used in the power calculation was 0.8%, a significant difference would therefore not be expected to be seen with the actual difference of 0.4% found in this study. However, the SD of the subjects in this study was considerably lower than initially estimated: 0.5 versus 1.3% used in the power calculation. If these new figures are used the actual number of subjects required to detect a significant difference of 0.4% is 16. The study was therefore well powered and able to detect a difference of 0.4%.

The reductions in HbA1c seen in this trial are comparable to previous trials with both ginseng and konjac mannan. A study with type 2 diabetes using konjac mannan fibre, showed a significant reduction in fructosamine over a three week period of about 0.19mmol/L which represent about a 6% reduction (Vuksan et al., 1999b). Similarly when ginseng was taken over a three-month period a reduction of
0.3% was seen in HbA1c (Vuksan et al., 2001b). In this study a reduction in HbA1c was observed of approximately 0.4%. Although this reduction is modest, the subjects in the study were well controlled at the start of the study with an average HbA1c level of 7%. This is the HbA1c treatment goal of diabetes. At the end of the study their average HbA1c levels were reduced to 6.5%, which, at the time of the study, was defined as normal. Similarly the reductions seen in total cholesterol and LDL cholesterol were modest, but again the subjects baseline values were low with almost a third of the patients on statin treatment. Nevertheless a 7% reduction of LDL cholesterol was achieved which translates in a 14% risk reduction in coronary heart disease (LRC, 1984). Furthermore, although not significant using the GLM repeated measures, both the Chol:HDL and LDL:Chol ratios decreased by 4 and 6% respectively when compared to control at week 12. A recent NCEP report which summarised the implications of the recent trials for the National Education Program Adult Treatment Panel III Guidelines (Grundy et al., 2004), concluded that these trials confirm the benefit of cholesterol-lowering therapy in high risk patients and support the ATP III treatment goal of LDL<100mg/dl (2.6mmol/L). Plotting relative risk for coronary heart disease against LDL-cholesterol, supports the concept “the lower the better” for reducing risk for major cardiovascular events is correct (figure 7.6). In this study the average LDL-cholesterol levels were reduced from 2.8mmol/L to 2.5mmol/L, and therefore decreased the average LDL level into the recommended range.

![Figure 7.6: Log linear relationship between LDL-cholesterol levels and relative risk for coronary heart disease (Grundy et al., 2004).](image)

Conjugated dienes in the LDL fraction are a marker of LDL oxidation. Therefore a reduction in conjugated dienes is an indicator of increased antioxidant activity and low levels are related to a reduced risk in cardiovascular disease (Holvoet et al., 2001). Reductions in LDL oxidation have been observed after feeding nuts, soy
and other vegetable protein diets (Ginsberg et al., 1998; Jenkins et al., 2002; Jenkins et al., 2001). In this study the conjugated dienes fell on both the control and treatment arms but this was not significant neither was there a significant difference between treatments.

HbAlc was the only variable which correlated with the amount of the supplements ingested. Both the total amount of supplement and VFB correlated with HbAlc but not ginseng, this fits in with other studies using viscous fibre. It was unfortunate that the OGTT data could not be used to calculate insulin sensitivity, as it is a more robust method than those which calculate insulin sensitivity using fasting levels only. Neither HOMA or QUICKI were significantly different between test and control.

It was hoped that the combination of VFB and ginseng would produce an additive effect and, although the trial was not designed to answer this question, this did not seem to be the case as the total reductions seen in either HbAlc or lipid did not seem to be substantially different from the results of previous trials which used only one or the other. However, the ginseng/VFB combination caused a small but clinically significant improvements in diabetes control and cardiovascular risk factors in this group of well-controlled subjects with diabetes and although small may be a useful adjunct in the armamentarium of strategies required to achieve the new, much lower, recommended treatment goals set for people with diabetes.
Chapter 8

GENERAL DISCUSSION

8.1 Introduction

The aim of the thesis was to determine whether synergistic or additive benefits could be derived with the use of two plant-based components with independent physiological benefits and whether the use of these two agents would improve diabetes control in the long term. Combination drug therapy to treat chronic disease is already common practice, pharmacological agents such as biguanide and sulfonylurea or biguanide and thiazolidinedione are now routinely used together in the treatment of type 2 diabetes (CDA, 2003). Combining dietary components to treat chronic disease is another approach which has been used, for example the recent "dietary portfolio diet", which uses an array of nutritionally active components such as soy protein, plant sterols, viscous fibre, and almonds, has been shown to be as effective at reducing LDL-cholesterol levels as a first generation statin (Jenkins et al., 2003).

If dietary components and so-called natural health products (plant derived nutraceuticals) are to have clinical relevance it is likely they will have to be used in combination since singly their effects are often small. This issue has taken on an even greater significance as the levels of risk factors considered acceptable, be it blood pressure control, glycaemic control or LDL-cholesterol are progressively reduced. Thus acceptable levels for blood pressure have been reduced from 140/90 to 130/85 mmHg (Grundy et al., 2004), HbA1c from 7.0 to 6.0% (CDA, 2003), and now for high risk individuals, which includes diabetes, it has been suggested that LDL-cholesterol should be reduced from 2.5 mmol/L to less than 1.8 mmol/L (Grundy et al., 2004). It is in this context that our studies may be of use since even small effects may benefit those on the borderline of the range. Furthermore, in view of the pleiotropic effects now looked for in drugs such as statins, using a combination of natural products may also have value.
Individually both American ginseng and konjac mannan have been shown to improve metabolic control in healthy and diabetic individuals. Acute studies with American ginseng (AG) have demonstrated reductions in postprandial glucose levels (Vuksan et al., 2000a), these were accompanied by increases in the early phase of insulin secretion (Vuksan et al., 2001b). Long term studies with AG in type 2 diabetes have confirmed the possible benefits of this substance with reductions in HbA1c and blood pressure (Vuksan et al., 2001b). Viscous fibre too has been shown to reduce postprandial glucose levels (Jenkins et al., 1977), however these reductions have been associated with decreases in postprandial insulin and GIP (Jenkins et al., 1980; Morgan et al., 1990). Early work with guar gum in the 1970's demonstrated the importance of viscosity (Jenkins et al., 1978). Since the 1970's several other viscous fibres such as oat bran and psyllium have been found to effective in the treatment of diabetes and hypercholesterolaemia (Anderson et al., 2000; Anderson et al., 1991). One of the most viscous fibres coming from the Orient is konjac mannan fibre and has been shown to improve metabolic control in type 2 diabetes and improve systolic blood pressure (Vuksan et al., 1999b). Therefore, as both AG and konjac have been shown to improve diabetes control through independent physiological mechanisms, the next logical step was to explore the possible benefits of using these in combination.

8.2 Acute Studies

Two of the objectives for the acute studies, as outlined in Chapter 1, were to establish the effect of konjac mannan and ginseng alone and in combination on postprandial glycaemia, insulinaemia, and gastrointestinal hormone secretion in type 2 diabetes. The hypothesis was that the two compounds would act synergistically and the acute day-profile study (Chapter 4) was designed to answer this question. However the results were less than clear. Neither the ginseng or the konjac when given individually changed postprandial glucose and insulin levels by the expected amounts and the combination did not result in glucose levels significantly different from control. This study did demonstrate that konjac mannan fibre flattens the postprandial response of both GIP and GLP-1, an effect not previously demonstrated. The reduction in the postprandial response of GIP and GLP-1 after fibre confirms again the proposed mechanism of action of fibre i.e. the reduced rate of nutrient absorption from the gastrointestinal tract. The inability to demonstrate a significant
effect of either ginseng or konjac on postprandial glucose levels may have been due to several factors: an ineffective ginseng preparation, lack of sufficient gelling of the konjac fibre to deal with the carbohydrate load, and finally, the study may have been underpowered. The combination of ginseng and konjac did not seem to have any added benefit over that of either ginseng or konjac by itself. However due to the subsequent findings that the ginseng used was not physiologically active, and that the mode of konjac incorporation interfered with the forming of a viscous solution, made it difficult to draw firm conclusions on the possible additive effect of ginseng and konjac. At the time these results became available we were the recipients of additional grant money to explore the long-term effects of AG and konjac used in combination. Considerable time and money was needed to find physiologically effective sources of both konjac and ginseng. Once these sources had been identified no further resources were available to explore the combined acute effects in more detail. However this could be the subject of future work.

The third objective was to establish the effect of konjac and ginseng on the postprandial glucose and insulin response to a second meal. Previously it has been shown that another viscous fibre, guar gum, flattened the postprandial glucose response to a second meal. Konjac was therefore expected to have a similar effect. Although there were no significant differences in glucose response at any time point, the study did show a reduction in the incremental areas under the glucose curve of the second meal after the konjac containing meals. This effect on the second meal may play an important role in the effectiveness of high fibre diets over the long term where consumption of fibre with each meal might not be practically possible.

The studies described in this thesis again highlight the problems when working with “natural products”. Many variables including, the specific species used, growing conditions, processing of the raw material, method of administration etc can all influence the physiological effect of the compound. Even though the ginsenoside content of the ginseng used in the day-profile study was one of the highest, the effect on glycaemia was minimal. The only way to make sure at this point in time that a ginseng is effective is to test it in vivo. Further work could explore the individual active components of the ginseng. Similarly, the experiments with konjac mannan highlighted the importance of both the method of its incorporation into food and the importance of viscosity. The effectiveness of the konjac when mixed into margarine vs bread was
unexpected, as the traditional way of incorporating fibre has always been to mix it with the carbohydrate portion of the meal. The effectiveness of the konjac when mixed with fat could be related to a slowing of gastric emptying where, due to the lack of separation of the fat and liquid phases in the stomach, a dispersion would be formed which may slow the rate of gastric emptying. Slowing of gastric emptying would reduce the rate of glucose absorption in the small intestine. Future studies could explore the reason for the effectiveness of the viscous fibre/fat combination. However, this method of incorporation suggests a very different range of products or possible methods of incorporating viscous fibre.

The final objective of the thesis was to establish the effect of using viscous fibre and ginseng on metabolic control in a long-term trial in type 2 diabetes.

8.3 Long Term Study

In the long-term study (Chapter 7), subjects consumed approximately 6g of a viscous fibre blend (VFB) and 2.5g of ginseng for three months. Significant improvements in HbA1c, total cholesterol, LDL-cholesterol, and ApoB were observed when compared to the control treatment. The improvement in lipid metabolism was estimated to represent a 14% reduction in cardiovascular risk. Viscous fibre and possibly ginseng might therefore be useful additions to the armamentarium necessary to reduce cardiovascular risk factors. The reductions in HbA1c and lipids seen in this trial were comparable to previous trials with ginseng and konjac mannan used individually, and therefore there seemed to be again no indication of an additive or synergistic effect of these two compounds. It is possible that the different mechanisms of action of viscous fibre and ginseng are not compatible. The flattening of the postprandial insulin response by viscous fibre might negate the positive benefits of the incretin effect of the ginseng. Indeed the results from both the acute and long-term studies seem to indicate that any benefit seen was due to the presence of the viscous fibre rather than the presence of ginseng.
8.4 Concluding Remarks

The use of viscous fibre, functional foods and the glycaemic index are now recognised in the effort to lower cholesterol levels (Grundy et al., 2004; Sheard et al., 2004). Both ginseng and konjac are potentially useful compounds to be used in reaching target cholesterol levels. The potential incretin effect of AG and the highly viscous nature of konjac would seem to make these ideal candidates to be used in combination. However the studies in this thesis seem to indicate that no additive effect is present when used in combination. The studies in this thesis further illustrate some of the pitfalls with “natural” product but also the potentially effective use of these compounds.
References


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Appendix

Published Papers
Appendix

Published Papers

Research Articles:


Reviews


ABSTRACTS:


**Editorial**