NOTE TO USERS

PLANT STEROLS AND GLUCOMANNAN AS HYPOCHOLESTEROLEMIC AND HYPOGLYCEMIC AGENTS IN SUBJECTS WITH AND WITHOUT TYPE 2 DIABETES

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Effects of Plant Sterols and Glucomannan on Lipid Profiles and Glycemic Control

ABSTRACT

The objective of this research was to examine the effects of plant sterols and glucomannan on lipid profiles, plasma plant sterol levels and glycemic control in mildly hypercholesterolemic subjects. Thirteen type 2 diabetic and sixteen non-diabetic individuals participated in a randomized crossover trial consisting of 4 phases, of 21 days each. During the study period, subjects were supplemented with plant sterols and/or glucomannan. Overall reductions of total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations were greater after consumption of plant sterols and glucomannan compared to plant sterol or glucomannan supplementation alone. Plasma lathosterol levels, indicators of cholesterol biosynthesis, were decreased after combination treatment. The results suggest that a combination of glucomannan and plant sterols substantially improve plasma lipids by reducing cholesterol absorption and synthesis simultaneously. Supplementation of plant sterols and glucomannan can thus be used as an effective treatment for management of circulating cholesterol levels and prevention of cardiovascular disease.

RÉSUMÉ

Cette étude avait pour objectif d'examiner l'effet d'un traitement composé de phytostérols et de glucomannan sur le profil lipidique, les concentrations de stérols dans le plasma ainsi que le contrôle glycémique chez des individus avec une hypercholestérolémie moyenne. Treize individus avec du diabète de type 2 et seize sans diabète ont participé à une étude croisée, dont l'ordre de traitement était déterminé au hasard. L'étude comportait 4 phases d'une durée de 21 jours chacune. Au cours de chaque phase, les participants recevaient un supplément de phytostérols et/ ou de glucomannan. Dans l'ensemble, les réductions de cholestérol total et de lipoprotéines de faible densité (LDL) étaient plus importantes suite à la consommation du mélange contenant les phytostérols et le glucomannan par rapport à la consommation individuelle de ces deux composés. Le niveau de lathostérol dans le plasma, indicateur de biosynthèse du cholestérol, montrait une diminution suite au traitement combiné. Ces résultats suggèrent que la combinaison de phytostérols et de glucomannan améliore la concentration de lipides plasmatiques de manière considérable. De plus, le traitement combiné semble réduire l'absorption et la biosynthèse du cholestérol simultanément. Il est conclu que la combinaison de phytostérols et de glucomannan pourrait servir de supplément pour traiter et gérer des niveaux de cholestérol élevés afin de prévenir des maladies cardiovasculaires.

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TABLE OF CONTENTS

LIST OF TABLES

Chapter 3. Manuscript

LIST OF FIGURES

Chapter 3. Manuscript

LIST OF ABBREVIATIONS

ABC ---------- ATP-binding cassette

apo ---------- Apolipoprotein

BMI ---------- Body mass index

CVD ---------- Cardiovascular diseases

HDL ---------- High-density lipoprotein

HMG-CoA -- 3-hydroxy-3-methylglutaryl coenzyme A

LDL ---------- Low-density lipoprotein

NCEP --------- National Cholesterol Education Program

VLDL --------- Very low-density lipoprotein

CONTRIBUTION OF AUHORS

In this thesis Chapter 3 is a manuscript, of which I am the primary author. The manuscript is based on my research conducted for my Master's thesis. It is co-authored by thesis supervisor, Dr. Peter Jones, physician Dr. William Parsons who conducted physical examinations of the participating subjects during the entire study period, Ms. Catherine Vanstone who assisted conducting the clinical trial, and Dr. Jerzy Zawistowski from Forbes Medi-tech Inc. who also contributed in the planning of this research project.

The idea and experimental components of this manuscript are a collection of inputs from Dr. Jones, Dr. Parsons, Ms. Vanstone, Dr. Zawistowski, and myself. I was responsible for the execution of the clinical study, data analysis and manuscript preparation. Dr. Jones and Ms. Vanstone provided assistance in the preparation and editing of the manuscript prior to submission for publication.

CHAPTER 1. INTRODUCTION

Dyslipidemia is frequently occurred in type 2 diabetic individuals. Although hypertriacylgriceridemia (Barrett-Connor *et al.* 1982), lowered high-density lipoprotein (HDL) cholesterol concentrations (Barrett-Connor *et al.* 1982), and increased small density low-density lipoprotein (LDL) cholesterol levels (Selby *et al.* 1993, Haffner *et al.* 1994) are major dyslipidemic symptoms in type 2 diabetes, hypercholesterolemia is also common (Kannel and McGee, 1979). Since elevated LDL cholesterol concentration is a risk factor of developing cardiovascular diseases (CVD), it is important to maintain lower circulating LDL cholesterol concentration (Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults, 2001) in individuals with elevated cholesterol levels.

Recently, plant sterols and glucomannan, a soluble dietary fiber, have been investigated individually as alternative remedies for hypercholesterolemia and hyperglycemia in both diabetic (Gylling and Miettinen 1996, Lee *et al.* 2003) and non-diabetic (Weststrate and Meijer, 1998, Vanstone *et al.* 2002) individuals. Plant sterols act as cholesterol-reducing agents, suppressing intestinal cholesterol absorption (Ikeda *et al.* 1989, Plat and Mensink, 1999, Nissinen *et al.* 2002), whereas glucomannan slows digestion (Doi, 1995), which suppresses hepatic cholesterol synthesis (Jones *et al.* 1993) via reduction in insulin secretion (Rodwell *et al.* 1976). Since plant sterols and glucomannan are known to reduce circulating cholesterol concentrations through different mechanisms, it is speculated that a combination of plant sterols and glucomannan reduces circulating cholesterol in a synergistic or additive manner.

In type 2 diabetics, increasing evidence suggests that cholesterol homeostasis is impaired along with the derangements in glucose and insulin control (Briones *et al.* 1986, Gylling and Miettinen, 1997). Whether the cholesterol lowering efficacy of plant sterols and/or glucomannan differ between type 2 diabetic and non-diabetic individuals, and whether alterations in cholesterol homeostasis are responsible for any differences, remains unknown.

Accordingly, aims of the present study are to (i) assess whether circulating lipid concentrations, non-cholesterol sterols, indicators of cholesterol kinetics, as well as glucose homeostasis respond to dietary supplementation of (a) plant sterols, (b) glucomannan, or (c) a combination of each in type 2 diabetic and non-diabetic individuals, (ii) to determine whether the degree of response of plasma lipid concentrations, non-cholesterol sterol profiles and glycemic control by supplementation of tested materials are different between type 2 diabetics and non-diabetics, and (iii) to examine if an interaction exists between changes in plasma lipid concentrations, non-cholesterol sterol profile and glycemic control between type 2 diabetic and non-diabetic groups and supplementation of plant sterols and/or glucomannan.

The overall goals of the study were addressed by testing the following null hypotheses:

1) The degree of response of circulating lipid concentrations, plasma non-cholesterol sterol profiles and glycemic control will not vary as a function of plant sterol and/or glucomannan consumption in individuals with and without type 2 diabetes.
2) The degree of response of circulating lipid concentrations, plasma non-cholesterol sterol profiles and glycemic control by consumption of plant sterols and/or glucomannan will be identical between type 2 diabetic and non-diabetic individuals.
3) There will be no interactive effect on circulating lipid levels, non-cholesterol sterol profile, and glycemic control between two subject groups (type 2 diabetic and non-diabetic group) and supplementation of plant sterols and/or glucomannan.

CHAPTER 2. LITERATURE REVIEW

2.1. Cholesterol Metabolism in Type 2 Diabetes

2.1.1. Alteration of cholesterol metabolism in type 2 diabetes

It has been suggested that cholesterol metabolism is altered in type 2 diabetic individuals. Compared to non-diabetic subjects, higher hepatic cholesterol synthesis and lower intestinal absorption rates have been reported in these patients (Briones *et al.* 1986, Gylling and Miettinen, 1997). Serum plant sterol levels, which are indicators of cholesterol absorption (Miettinen *et al.* 1990), are low in type 2 diabetics indicating reduced cholesterol absorption (Gylling and Miettinen, 1997). A similar phenomenon was also observed in non-diabetic individuals with higher blood glucose levels (Sutherland *et al.* 1992, Strandberg *et al.* 1996) and obese individuals (Miettinen and Gylling, 2000). In addition, bile acids, cholesterol synthesis rate, and cholesterol excretion are higher in individuals with type 2 diabetes compared to non-diabetic healthy controls (Bennion and Grundy, 1977, Briones *et al.* 1986, Gylling and Miettinen, 1997). This alteration in cholesterol homeostasis may account for the increased risk of CVD in type 2 diabetic individuals. The mechanisms for these alterations in cholesterol homeostasis remain unknown, however, many factors such as hyperglycemia (Naoumova *et al.* 1996), obesity (Miettinen, 1971, Nestel *et al.* 1973), insulin resistance (Naoumova et al. 1996, Simonen *et al.* 2002b), and hyperinsulinemia (Naoumova et al. 1996, Simonen *et al.* 2002b), are believed to contribute to the altered cholesterol homeostasis in type 2 diabetics.

2.1.2. Obesity and type 2 diabetes

The prevalence of diabetes parallels the increase in obesity, which is considered to be responsible for more than 75% of type 2 diabetes cases (Bennett, 1996). Recent population-based studies found that the prevalence of type 2 diabetes was directly correlated with an increased body mass index (BMI) (Wannamethee and Shaper, 1999, De Pablo-Velasco *et al.* 2002, Jia *et al.* 2003). An *in vitro* study showed that insulin sensitivity, which includes hepatic expression and protein phosphorylation of insulin receptors and insulin receptor substrate isoforms, was reduced in the obese mouse (Weigman *et al.* 2003). Thus, it is clear that obesity increases risk for the development of type 2 diabetes.

2.1.3. Obesity and cholesterol metabolism in type 2 diabetes

Obesity is also an independent risk factor for alterations in cholesterol homeostasis (Miettinen, 1971, Nestel *et al.* 1973). Several studies have reported that obese individuals have lower cholesterol absorption efficiency (Miettinen and Kesaniemi, 1989, Miettinen and Gylling, 2000) and higher cholesterol synthesis (Miettinen, 1971, Angelin *et al.* 1982) than lean individuals. Miettinen and Gylling (2000) observed lower dietary cholesterol absorption efficiency and an increase in serum and biliary cholesterol precursors in obese subjects compared with lean subjects and found that dietary cholesterol absorption was negatively associated with biliary concentrations of cholesterol. It is speculated that increases in the secretion of biliary cholesterol dilute dietary cholesterol levels in the large intestine and reduce the incorporation of dietary cholesterol into the micellar phase, thus inhibiting the amount of cholesterol absorbed (Miettinen and Gylling, 2000). Therefore, increases in biliary cholesterol secretion have

been proposed as a possible mechanism explaining the reduced cholesterol absorption efficiency in obese individuals.

LDL apolipoprotein (apo) B turnover is also enhanced in obese individuals possibly due to up-regulation of hepatic LDL apo B receptor activity (Kesaniemi and Grundy, 1983). Angelin *et al.* (1982) reported that the activity of the rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, was increased in the liver of obese subjects. These biochemical changes observed in obese individuals offer an explanation for their higher rates of cholesterol synthesis. Since obesity is common amongst people with type 2 diabetes, this likely contributes to the alterations in cholesterol homeostasis seen in these patients.

The effect of weight reduction in type 2 diabetic individuals on cholesterol homeostasis has recently been addressed. Studies of long-term and short-term weight reduction have resulted in improved cholesterol absorption efficiency, down-regulation of cholesterol synthesis, as well as the reduction of serum insulin and glucose levels in obese type 2 diabetics (Griffin *et al.* 1998, Simonen *et al.* 2000, Simonen *et al.* 2002a). Thus, obesity may contribute to the development of both insulin resistance and alterations in cholesterol metabolism in type 2 diabetic individuals.

However, regardless of obesity there is a direct association between the diabetic condition and the alterations seen in cholesterol metabolism (Naoumova *et al.* 1996, Simonen *et al.* 2002b). It has been shown that obese type 2 diabetic subjects have lower absorption and higher biosynthesis of cholesterol compared with obese non-diabetic individuals

(Simonen *et al.* 2002b). Neutral sterol and bile acid excretion and cholesterol turnover also tended to be higher in the type 2 diabetic group when compared with the non-diabetic group. Blood glucose level was positively correlated to fecal neutral sterol excretion in both groups. It was observed in another study that plasma mevalonic acid level, an index of whole body cholesterol synthesis, was higher in non-obese type 2 diabetics compared with age-matched non-diabetic subjects in response to hyperinsulinemia (Naoumova *et al.* 1996).

Taken together, it is evident that the obese condition contributes to the development of type 2 diabetes and an alteration in cholesterol homeostasis, however, the diabetic condition is independently associated with the altered cholesterol metabolism seen in type 2 diabetic individuals.

2.1.4. Insulin resistance, hyperinsulinemia, and cardiovascular disease

It is well established that the majority of patients who eventually develop type 2 diabetes have insulin resistance. Frequently, hyperglycemia occurs when these patients no longer sustain the degree of compensatory hyperinsulinemia required to maintain glucose homeostasis (McLaughlin and Reaven, 2003). Insulin resistance and hyperinsulinemia independently increase the risk for coronary artery disease and are correlated with increased frequency of myocardial infarction (Hsueh and Law, 1998). Insulin resistance is associated with a three-fold higher risk for developing coronary artery disease (Zavaroni, 1999). Furthermore, a population based study showed that higher fasting insulin levels were associated with increased risk of ischemic heart disease after adjusting for other

confounding factors, such as systolic blood pressure, medication, and family history of ischemic heart disease (Lamarche *et al.* 1998).

2.1.5. Insulin and cholesterol metabolism

Results of *in vitro* studies have shown that insulin can suppress intestinal cholesterol absorption and increase de novo cholesterol biosynthesis. Cholesterol uptake in the jejunum was shown to be enhanced in diabetic rats with a deficiency of insulin production (Thompson, 1980), and was normalized by supplementation of exogenous insulin (Thompson and Rajotte 1984). Other studies have shown that insulin stimulates cholesterol biosynthesis in isolated hepatocytes (Devery and Tomkin 1986) and cultured mammalian cells (Bhathena and Grundy, 1974). Higher postprandial cholesterol synthesis was also observed in both type 2 diabetic and non-diabetic individuals with a high postprandial insulin response compared to those with a low postprandial insulin response (Griffin *et al.* 1998).

Conversely, other studies have supported the potential role of insulin in suppressing cholesterol synthesis by increasing LDL receptor activity. *In vitro* studies have shown that high concentrations of insulin stimulate LDL receptor mRNA levels and activity in Hep-G2 cells (Wade *et al.* 1988, Wade *et al.* 1989). Mazzone *et al.* (1984) reported that LDL receptor activity and LDL uptake was enhanced after a 4-hour insulin injection. In a clinical study, hyperinsulinemia induced by acute injection of glucose in healthy male subjects showed a reduction in circulating levels of mevalonic acid, the immediate product of HMG-CoA reductase in the cholesterol pathway (Lala *et al.* 1994). Therefore, insulin may increase internalization of LDL and reduce HMG-CoA reductase activity

resulting in a decrease in cholesterol biosynthesis (Goldstein and Brown, 1990, Lichtenstein and Jones, 2001). Naoumova *et al.* (1996) conducted a clinical study involving non-obese subjects with and without type 2 diabetes. The results were consistent with the findings reported by Lala *et al.* (1994) that acute hyperinsulinemia decreases mevalonic acid levels. The reduction of mevalonic acid was less in the diabetic group, however, than in the non-diabetic group.

Overall, the role of insulin on cholesterol homeostasis has not been fully determined, but the regulatory signal by insulin in cholesterol metabolism may be impaired due to reduced insulin sensitivity associated with the diabetic condition. Thus, improvement of insulin sensitivity seems to be essential to normalize cholesterol homeostasis in diabetic individuals.

2.2. Plant Sterols

2.2.1. Structure of plant sterols

Plant sterols are structurally similar to cholesterol, with exception of C-24 position on the sterol side chain. Vegetable oils are the most concentrated source of plant sterols in the North American diet. Small amounts of plant sterols are also found in nuts, grains, and whole vegetables (Ostlund Jr., 2002). Estimated dietary intake of plant sterols varies from 170 to 440 mg/day in various populations (Ahrens Jr. and Boucher, 1978, Cerqueira *et al.* 1979, Hirai *et al.* 1982, Miettinen and Kesaniemi, 1989, Morton *et al.* 1995). The most abundant forms of sterols in foods are campesterol, β-sitosterol, and stigmasterol (Clifton, 2002, Ostlund Jr., 2002). Reduction of the double bond of sterols at the 5α position by

hydrogenation leads to the formation of plant stanols, such as campestanol and sitostanol (Ling and Jones, 1995) Plant sterols are not synthesized and poorly absorbed in humans. Two ATP-binding cassette (ABC) transporters, ABCG5 and ABCG8, have been found to be implicated in sterol absorption (Berge *et a*l. 2000, Lee *et al.* 2001, Lu *et al.* 2001). These transporters pump absorbed plant sterols and cholesterol selectively from the enterocytes back into the intestinal lumen thereby regulating absorption rates. It has been reported that cholesterol absorption is 56.2 ± 12.1% in normal subjects (Bosner *et al.* 1999), while only 2-5% of plant sterols are absorbed (Ostlund Jr., 2002). Accordingly, levels of sitosterol and campesterol in human plasma are approximately 0.1-0.14% of those of cholesterol (Miettinen *et al.* 1990).

2.2.2. Plant sterols as a cholesterol-lowering agent

Plant sterols have been considered as cholesterol-lowering agents since the early 1950's (Pollack, 1953). The cholesterol-lowering efficacy of plant sterols has now been unquestionably established (St-Onge and Jones, 2003). The American Heart Association and National Cholesterol Education Program (NCEP) have made an intake recommendation of 2 g/day of plant sterols or stanols in combination with a healthy diet to lower blood cholesterol levels (Krauss *et al.* 2000, Expert panel on detection, elevation and treatment of high blood cholesterol in adults. 2001). In addition, the US Food and Drug Administration has approved the hypocholesterolemic health claim to be placed on plant sterol enriched products (Schaefer, 2002).

Earlier studies focused on the action of β-sitosterol and sitostanol in reducing circulating total and LDL cholesterol levels (Becker *et al.* 1993, Denke, 1995, Jones *et al.* 1999). Due to extremely low solubility, plant sterols and stanols are often esterified with fatty acids to form sterol/stanol esters which increase their fat solubility and their ability to be incorporated into foods, such as margarines and dressings. Cholesterol-lowering efficacy of plant sterol and stanol esters has been extensively investigated (Hallikainen *et al.* 2000a, Jones *et al.* 2000). Recent studies have shown that plant sterol esters, stanol esters, and free sterol/stanol mixtures all have equal cholesterol-lowering effects (Weststrate and Meijer, 1998, Hallikainen *et al.* 2000a. Jones *et al.* 2000, Miettinen *et al.* 2000, Clifton, 2002, Vanstone *et al.* 2002). No difference in hypocholesterolemic efficacy between sterol and stanol ester-enriched spreads has been demonstrated (Weststrate and Meijer, 1998, Hallikainen *et al.* 2000a, Clifton, 2002, Vanstone *et al.* 2002). This finding contradicts the results of a previous trial (Becker *et al.* 1993) where stanols were found more effective than sterols in decreasing circulating cholesterol concentrations. The discrepancy between these results has been thought to be due to different approaches of incorporating plant sterols and their derivatives into diets. For example, 3 g/day of stanol given in capsules showed no cholesterol-lowering effect on plasma lipid levels (Denke, 1995). Diet differences may also cause the discrepancy in the degree of total and LDL cholesterol reductions across various mixtures examined (Hallikainen and Uustiupa, 1999, Jones *et al.* 1999). In a recent meta-analysis of 14 studies examining plant sterol and stanol supplements in margarines and spreads, Law (2000) concluded that ~ 2 g of plant sterols or stanols lower serum LDL cholesterol concentrations by 9-14%, with little or no effects on HDL cholesterol or triglyceride concentrations. Cholesterol-lowering efficacy of plant sterols and stanols has also been observed in familial

hypercholesterolemic (Becker *et al.* 1993, Gylling *et al.* 1995, Amundsen *et al.* 2002) and normocholesterolemic children (Special Turku Coronary Risk Factor Project; Tammi *et al.* 2002).

2.2.3. Plant sterols and type 2 diabetes

Alteration of cholesterol metabolism has often been reported to be associated with hypercholesterolemia in type 2 diabetic subjects. Gylling and Miettinen (1996) examined the effectiveness of plant stanol ester combined with statin treatment on cholesterol reduction in 8 mildly hypercholesterolemic type 2 diabetics. The results showed that plant stanol ester (3 g/day) and statin (40 mg/day) treatment for 7 weeks reduced total cholesterol, LDL cholesterol, and apo B lipoprotein by 35%, 44 %, and 45%, respectively. Recently, Lee *et al.* (2003) conducted a randomized, placebo-controlled, double-blinded clinical trial involving 85 type 2 diabetics to determine the efficacy of plant sterols on lowering cholesterol absorption. Subjects consumed 0.8 g of plant sterol esters twice per day with their usual diet over 12 weeks. After 4 weeks, total and LDL cholesterol were reduced by 5.2% and 6.8%, respectively, in the plant sterol group compared to baseline. However, the reductions became smaller and were not significant compared to baseline after 8 and 12 weeks. Since body weight and medication were maintained during the intervention, the alleviated reduction of circulating cholesterol concentrations in plant sterol treated group may be due to poor compliance for plant sterol supplementations under free-living conditions in a long-term study.

2.2.4. Hypocholesterolemic mechanisms of plant sterols

In vitro and *in vivo* studies in animals showed that plant sterols compete with cholesterol for bile salts and phospholipids in forming micelles, thus reducing cholesterol absorption (Ikeda *et al.* 1988a, Ikeda *et al.* 1988b, Ikeda *et al.* 1989, Plat and Mensink, 1999). Because of their hydrophobic nature, plant sterols and stanols have a greater affinity for micelles than does cholesterol (Armstrong and Carey, 1987). In addition, studies in humans and animals (Child and Kuksis 1986, Nissinen *et al.* 2002) have suggested that plant sterol and stanol esters cause reductions in the solubility and hydrolysis of cholesterol esters in the small intestine. In a recent study (Nissinen *et al.* 2002), it was shown that when intestinal plant stanol concentration is high, micellar solubility of cholesterol is reduced, possibly due to the replacement of its free fraction by hydrolyzed plant stanols.

The inhibition of intestinal cholesterol absorption is partially compensated for by an increase in cholesterol hepatic synthesis rate (Jones *et al.* 2000, Miettinen *et al.* 2000). It was observed that after the ingestion of sterol and stanol ester containing diets for 3 weeks, cholesterol absorption was reduced by 26-36% (Jones *et al.* 2000). Although endogenous cholesterol biosynthesis was increased by 37-53% in sterol and stanol ester diet groups, the total and LDL cholesterol levels were still lower than controls. Similar results were reported by Gylling *et al.* (1999a), in that serum cholesterol precursor sterol concentrations, an indicator of cholesterol biosynthesis rate, were raised by 10-46% after stanol ester intake, indicating that cholesterol synthesis was increased. Increased concentrations of serum lathosterol, cholesterol precursor sterol, were also observed in subjects after consumption of plant stanol esters for 8 weeks (Plat and Mensink 2002a).

Plat *et al.* (2000) examined the effects of 2.5 g/day of plant stanols, consumed either once per day or divided into three doses per day, on LDL cholesterol levels in thirty-nine healthy normocholesterolemic or mildly hypercholesterolemic subjects. Results showed that it was not necessary to consume plant stanols at each meal or simultaneously with dietary cholesterol to obtain the maximal cholesterol-lowering effect. Replacement of intestinal cholesterol from the micelles may not be the only mechanism by which plant sterols lower cholesterol absorption. A recent study showed that plant sterols increased LDL receptor expression as measured by RNA and protein levels by 25-43%, and the change in LDL receptor expression was negatively associated with LDL cholesterol levels (Plat and Mensink, 2002a). Therefore, it is reasonable to speculate that the cholesterol-lowering effect of plant sterols is also due to increases in the clearance of LDL cholesterol from the circulating system.

In addition to enhanced clearance of LDL cholesterol from the circulation, it was reported that the ABC transporter A1, a membrane bound protein, is involved in the mechanism of suppression of cholesterol absorption by plant stanols (Plat and Mensink, 2002b). In the small intestine, ABC A1 is thought to facilitate the transport of cholesterol from enterocyte into the lumen (McNeish *et al.* 2000, Repa *et al.* 2000). It was shown that mixed micelles enriched with sitostanol or with cholesterol and sitostanol increases ABC A1 expression in caco-2 cells (Plat and Mensink, 2002b), an accepted model to study human intestinal lipoprotein metabolism (Levy *et al.* 1995). Therefore, it has been hypothesized that plant stanols and possibly sterols reduce cholesterol absorption by

enhancing ABC A1 mediated cholesterol efflux back into the intestinal lumen (Plat and Mensink, 2002b).

2.2.5. Dose-response of plant sterols on lipid profile

A dose-response effect of plant sterols on circulating cholesterol concentrations has been investigated extensively. In a randomized crossover trial, different doses of sterol esters ranging from 0.83 to 3.24 g/day were given to normocholesterolemic subjects for 3.5 weeks (Hendriks *et al.*1999). Results showed that the reductions in total and LDL cholesterol levels were correlated with the administered doses up to a dose of 1.6 g/day, with no further significant decrease in the circulating cholesterol observed thereafter. Similar results have been reported by Hallikainen *et al.* (2000b) who examined the dose-response effect in hypercholesterolemic individuals. Recently, Law (2000) reviewed 14 randomized double blind trials utilizing plant sterol and stanol spread products in adults. There was a dose-response effect up to 2 g of plant sterol or stanol per day, which reduced LDL-cholesterol by 0.4 to 0.5 mmol/L. A similar observation was reported by Ostlund Jr. (2002). A measurable hypocholesterolemic effect was seen with 900 mg/day plant sterol and stanol supplementations, and reduction was nearly maximal (9.6% lowering) at a dose of about 2 g/day, which is the recommended dose by the NCEP for a cholesterol-lowering alternative (Expert panel on detection, elevation and treatment of high blood cholesterol in adults. 2001). Recently, it was observed that 328 mg/day of plant sterols lowered the efficiency of cholesterol absorption (Ostlund Jr. *et al.* 2003). Overall, it is evident that 1.6-2.0 g/day of plant sterols and stanols provide the greatest cholesterol-lowering effect on general population.

2.2.6. Side effects of plant sterols

A major safety concern of plant sterol supplementation is in individuals with a rare genetic disorder termed phytosterolemia. This condition is caused by a mutation in the ABC proteins, ABC G5 and ABC G8, which are expressed in intestine and liver (Berge *et al.* 2000). It has been hypothesized that ABC transporters G5 and G8 are able to discriminate between cholesterol and plant sterols (Bhattacharyya and Connor, 1974, Salen *et al.*, 1992, Lütjohann and von Bergmann, 1997) and they pump plant sterols out of the intestinal cell back into the gut lumen (Allayee et al. 2000, Chen, 2001). However, because of the mutation in the ABC G5 and ABC G8, an increased absorption of plant sterols occurs, and high concentrations of plasma sterols have been observed. Consequently, patients with phytosterolemia are more likely to develop atherosclerosis. Thus, individuals with phytosterolemia should avoid taking plant sterol-fortified products.

Plasma sterols and stanols inhibit intestinal cholesterol absorption, but may also interfere with the absorption of fat-soluble vitamins and vitamin precursors. Because hydrophobic compounds are transported by lipoproteins, the reduction in the number of LDL particles in the circulation after consumption of plant sterols or stanols may affect plasma concentrations of fat-soluble vitamins and their precursors such as carotenoids and tocopherols. For this reason, these fat-soluble vitamin levels are often standardized to plasma lipids or cholesterol concentrations (Kerckhoffs *et al.* 2002). Changes in fat-soluble vitamin concentrations have been assessed in many clinical studies involving plant sterols and stanols.

An earlier study by Weststrate and Meijer (1998) investigated the effects of 1.5-3.3 g/day of plant sterols and stanol-esters on the absorption of fat-soluble vitamins. Ninety-five subjects consumed plant sterol and stanol-ester enriched margarine for 3.5 weeks. Results showed that lipid-standardized plasma α- and β-carotenoids were decreased in both treatments. However, plasma lycopene levels were not affected. Another short-term study (Hendriks *et al.*1999) showed plasma lipid standardized concentrations of α- and β-carotene and lycopene were reduced by 15% and 19%, respectively, with a higher dose of 3.24 g/day plant sterols. β-carotene reductions have also been reported in a long-term (Gylling *et al.* 1999b) and two short-term (Hallikainen *et al.* 1999, Mensink *et al.* 2002) studies using sitostanol-enriched margarine. Amundsen *et al.* (2002) reported small changes of serum carotenoids in children after the consumption of plant sterols. Lipid standardized concentrations of α- and β-carotene were reduced by 11% and 8%, respectively by the end of the intervention periods, but these changes were not significant after adjusting for baseline differences (Amundsen *et al.* 2002). Another study by Raeini-Sarjaz *et al.* (2002) observed no changes of α- and β-carotenoids in hypercholesterolemic subjects after the consumption of 1.8 g/day esterified plant sterols and stanols for 3 weeks. While Gylling *et al.* (1999b) found that the intake of sitostanol esters affects absorption of tocopherols and β-carotene. Similarly, Hallikainen *et al.* (2000b) showed a dose dependent reduction of serum α- and β-tocopherol concentrations with the consumption of plant stanol esters (0.8-3.2 g/day) for 4 weeks. However, in both studies after lipid-adjustment, the changes in tocopherols were not significant. Another study in children by Amundsen *et al.* (2002) observed an increase of α-tocopherol (7.1%) and retinol (15.6%) after lipid-adjustments. Serum concentrations of 25-hydroxyvitamin D, retinol, and vitamin K were not affected in the same studies in which carotenoids were

reduced (Gylling and Miettinen, 1999, Hendriks *et al.* 1999, Plat and Mensink, 2001). Plat and Mensink (2001) observed that the reduction of carotenes and lycopenes were related to the decrease of LDL cholesterol levels.

Overall, most studies have demonstrated decreases in plasma carotene levels, however, the results tend to be inconsistent. This discrepancy among findings may be due to the varied length of treatment period, composition, or dosage of plant sterol mixtures, and diet.

Several animal studies have indicated side effects after administration of plant sterols and stanols. Increased concentrations of plant sterols in erythrocyte membranes may result in their increased fragility. In an earlier study in rats, Leikin and Brenner (1989) reported that membrane rigidity was increased in liver microsomes enriched with β-sitosterol and campesterol. An *in vitro* study showed that high β-sitosterol levels (up to 0.7 mmol/L) can cause contraction of human umbilical vein endothelial cells (Boberg *et al.* 1991). These observations suggested that high plasma concentrations of β-sitosterol may have potentially cytotoxic effects, and it may interfere with cellular functions. In addition, a systematic review of *in vitro* and *in vivo* studies by Moghadashian (2000) concluded that high concentrations of plant sterols in the plasma may affect reproductive organs in laboratory animals. However, all of the adverse effects previously discussed are based on animal studies in which much higher doses of plant sterols have been applied as compared to human studies. Moreover, the absorption of plant sterols has been reported to be lower in humans, (Miettinen *et al.* 1990) with the exception of individuals with

phytosterolemia. It is suggested that consumption of plant sterols is relatively safe in humans (Ostlund Jr., 2002).

2.3. Glucomannan

2.3.1. Characteristics of glucomannan

Glucomannan is the generic term for a water-soluble dietary fiber obtained from the tubers of *Amorphophallus konjac*, and is a traditional food in Asia. Glucomannan is tasteless and odorless. Purified glucomannan is a polysaccharide which consists of repeating units of β-D-glucose and β-D-mannose joined together in a chain by 1,4 linkage (Yui *et al.* 1992). Glucomannan has been acknowledged as both a cholesterol-lowering (Haskell *et al.* 1992) and hypoglycemic agent (McCarty, 2002). The structural properties of glucomannan are important determinants of its hypocholesterolemic and hypoglycemic abilities (Arvill and Bodin, 1995). Glucomannan particles consist of extremely long threadlike polysaccharides that tangle together. In conjugation with water, the volume of glucomannan particles can increase up to 200 times, and resulting in a very viscous liquid. The viscosity of 1% glucomannan solution gradually increases to over 10000 cps in an hour and reach a peak of 50000 cps after 5 hours (Maekaji, 1974, Kishida *et al.* 1978, Doi, 1995). High viscosity is thought to play the most important role for hypocholesterolemic and hypoglycemic effects of glucomannan (Vuksan *et al.* 2000, McCarty, 2002).

2.3.2. Glucomannan as a hypocholesterolemic and hypoglycemic agent

Several earlier and recent studies have observed the hypocholesterolemic efficacy of glucomannan using animal models. Hou *et al.* (1990) examined the hypocholesterolemic effect in rats after 12 weeks of glucomannan treatment. Male and female Sprague-Dawley rats were fed diets with three different glucomannan (2.5, 5, and 10%) concentrations. Results showed that glucomannan induced a marked reduction in cholesterol levels in serum and liver, and increased stool bulk. Hozumi *et al.* (1995) also observed reductions of both glucose and cholesterol concentrations in rats fed glucomannan for 18 weeks. A study assessed the effect of glucomannan on intestinal cholesterol absorption as well as fat and bile acid excretion in rats (Gallaher *et al.* 2000). Total cholesterol levels in liver were significantly higher in glucomannan groups compared to the cellulose and control groups. Cholesterol absorption measured by the fecal isotope ratio method was reduced by 20.2% in the glucomannan group relative to control.

The efficacy of glucomannan on hypocholesterolemic and hypoglycemic properties has been observed in clinical studies. An eight-week double-blind trial (Walsh *et al.* 1984) was conducted on 20 obese subjects to test purified glucomannan fiber as a food supplement (1g/day as two 500 mg capsules). Results showed a significant loss of body weight (5.5 lbs) and reductions in serum total and LDL cholesterol levels after glucomannan supplementation for 8 weeks. Gallarher *et al.* (2002) investigated the hypocholesterolemic effect of a supplement that contained equal amounts of glucomannan and chitosan. Twenty-one overweight normocholesterolemic subjects took 2.4 g/day of the supplement for 4 weeks. Although it was impossible to distinguish between the efficacy of glucomannan and chitosan, reduction in cholesterol levels was

observed after supplementation with this mixture. There was also a trend towards greater fecal excretion of neutral sterols and bile acids after consuming glucomannan and chitosan. This may partially explain the cholesterol-lowering mechanism of action of glucomannan.

The hypoglycemic efficacy of glucomannan has been investigated in non-diabetic and type 2 diabetic individuals. Hopman *et al.* (1988) reported that the addition of 2.6 g or 5.2 g of glucomannan to a carbohydrate rich breakfast decreased postprandial rise in plasma insulin in 8 patients with previous gastric surgery. Similar results were observed by Scalfi *et al.* (1987) using 6 g/day of glucomannan. Effects of several different fibers on glycemic control were compared in healthy non-obese subjects (Morgan *et al.* 1990). Six male subjects were given three test meals containing 10 g guar gum, 10 g sugar beet fiber, 10 g soya-bean-cotyledon fiber, or 5 g glucomannan on separate occasions. Circulating glucose levels were unaffected by the addition of either soya-bean-cotyledon fiber or glucomannan to the meal. However, postprandial insulin levels were lowered after glucomannan and guar-gum supplementations. The hypoglycemic effect of glucomannan was found in 72 type 2 diabetic subjects. Fasting blood glucose and 2 hour postprandial blood glucose were significantly reduced after both 30 days and 65 days of supplementation compared to baseline (Huang *et al.* 1990).

Several studies have examined changes in both circulating cholesterol levels and glycemic control. Doi *et al.* (1979) reported that fasting glucose levels and total cholesterol concentrations were reduced by 29% and 11%, respectively, in 13 type 2 diabetic subjects after supplementation of 7.8 g/day of glucomannan for 90 days. In

another study (Vuksan *et al.* 2000), 11 type 2 diabetic persons were treated with 8 to 13 g/day of glucomannan for 3 weeks. No reduction in insulin level was observed, however, fructosamine was reduced by 5.2%. Total and LDL cholesterol levels were decreased by 12.4% and 22%, respectively. The reason that Vuksan *et al.* (2000) were unable to observe a reduction in fasting insulin level may be due to either relatively short experimental period or longer baseline period (8 weeks) in which subjects followed an NCEP II diet, before the supplementation of glucomannan.

Recently, Chen *et al.* (2003) evaluated the effects of a glucomannan supplement (3.6 g/day) on blood lipids and glucose levels for 28 days in 22 hyperlipidemic type 2 diabetic patients. Total and LDL cholesterol, apo B, and fasting glucose were reduced by 11%, 20%, 12.9%, and 23.2%, respectively. Fecal sterol and bile acid concentrations were increased by 18% and 75.4%, respectively. The results of these studies suggest that glucomannan supplementation improves blood lipid levels by enhancing fecal excretion of neutral sterols and bile acids, and alleviates the elevated glucose levels in diabetic subjects by slowing carbohydrate digestion and absorption.

2.3.3. Hypocholesterolemic and hypoglycemic mechanism of glucomannans

It has been suggested that the high viscosity of glucomannan slows down the rate of nutrient absorption in the small intestine (Doi, 1995), which subsequently lowers the postprandial glycemic and insulinemic responses. An earlier study by Jenkins et al. (1978) compared the hypoglycemic efficacies of soluble fibers (guar gum and pectin) and insoluble fiber (wheat bran). Results showed that 12 g/day of soluble dietary fibers reduced blood glucose and insulin concentrations during a 50 g oral glucose tolerance

test. It was also found that the peak of glucose reduction was positively correlated with each fiber's viscosity. The magnitude of glycemic response was directly related to the viscosity and concentration of soluble fiber in the food tested (Wursch and Pi-Sunyer, 1997). Although glucomannan was not examined in this study, since glucomannan has a higher viscosity than the fibers administered in the study, glucomannan may improve glycemic control to a greater extent than other fibers (Ebihara and Schneeman, 1989). Reduction of blood glucose decreases insulin secretion, which in turn, decreases the activation of hepatic cholesterol synthesis (Jones *et al.* 1993), by suppressing insulin-induced HMG-CoA reductase activity (Rodwell *et al.* 1976).

Enhanced production of bile acids in liver, and fecal excretion of neutral and sterol and bile acids, have been considered to be possible mechanisms through which glucomannan may affect cholesterol levels (Vuksan *et al.* 2000). Cholesterol conversion to bile acids represents 50% of total elimination of cholesterol each day in humans. Studies in hamsters (Horton *et al.* 1994) and rats (Matheson *et al.* 1995) demonstrated that soluble fibers, psyllium and pectin, stimulate hepatic 7 α-hydoxylase activities, (the rate-limiting enzyme of bile acid synthesis) and consequently increased the synthesis of bile acid.

Studies have shown that short-chain fatty acid production from glucomannan by anaerobic bacteria in the colon may contribute to the hypocholesterolemic (Chen *et al.* 1984) and hypoglycemic actions of glucomannan (Cumming and Englyst, 1987). Chen *et al* (1984) observed that 0.5% sodium propionate supplement significantly decreased the serum and liver cholesterol in rats. Supporting data have been reported by Wright *et al.* (1990) that propionate reduced cholesterol synthesis rate of cultured hepatocytes. In a

clinical study, Venter *et al.* (1990) reported that propionate supplementation improved glucose tolerance and insulin sensitivity, and suppressed cholesterol synthesis by down-regulating HMG-CoA reductase activity. Propionate decreases cholesterol synthesis by inhibiting acetate utilization (Williamson *et al.* 1981). Results of these studies suggest that glucomannan may suppress cholesterol biosynthesis by supplying short-chain fatty acids through its fermentation in the colon.

2.3.4. Dose-response of glucomannan on lipid profile

A meta-analysis by Brown *et al.* (1999) showed a dose-response effect of water-soluble fibers in reducing blood cholesterol. It was found that the hypocholesterolemic effect of soluble fibers increases with the dose up to 10 g/day for total cholesterol level and up to 8 g/day for LDL cholesterol level. However, effects of glucomannan were not examined in this meta-analysis. Due to the limited number of studies, it is difficult to establish the dose-response of glucomannan for its cholesterol-lowering effect. Arvill and Bodin (1995) observed 10% and 7.2% of reductions in total and LDL cholesterol levels, respectively, in healthy subjects after consumption of 3.9 g/day of glucomannan for 4 weeks without diet control. Vuksan *et al.* (2000) reported 12% and 22% reductions in total and LDL cholesterol levels, respectively, after 3 weeks of supplementation with 8-13 g/day of glucomannan, consumed as biscuits together with an NCEP II diet. A recent study showed similar effects of glucomannan (3.6 g/day) on total and LDL cholesterol in hyperlipidemic type 2 diabetic subjects who followed the NCEP I diet (Chen *et al.* 2003). The degree of the hypocholesterolemic effect likely varies depending on the dietary intake of subjects, study population, and form of glucomannan.

2.3.5. Dose-response of glucomannan on glycemic control

It is not clear if the hypoglycemic effect of glucomannan is dose-dependent. It has been reported that a combination of 3.6 g/day of glucomannan supplement and NCEP I diet reduced by 23.3% fasting glucose concentrations in diabetic patients (Chen *et al.* 2003). In a recent review (McCarty, 2002) concluded that an administration of 4-5 g/day of glucomannan was able to slow carbohydrate absorption and suppress the rise of postprandial glucose and insulin levels. However, Vuksan *et al.* (2000) observed no change in serum glucose and insulin concentrations, even though a large dose of glucomannan (10-13 g/day) was administered to subjects with insulin resistance syndrome, possibly due to a longer run-in period during which subjects followed the NCEP II diet. Further investigations are needed to determine whether glucomannan affects cholesterol and glucose metabolism in a dose dependent manner.

2.3.6. Side effects of glucomannan

Early studies showed that glucomannan was well tolerated and produced no adverse effects in both non-diabetic and diabetic subjects (Doi *et al.* 1979, Arvill and Bodin, 1995). In these studies, 3.6 g/day (Doi *et al.* 1979) and 3.9 g/day (Arvill and Bodin, 1995) glucomannan was provided in capsules and taken by subjects before each meal. Similarly, in a recent study, 3.6 g/day glucomannan was administered for 4 weeks to diabetic patients (Chen *et al.* 2003) with only one subject experiencing minor gastric discomfort at the beginning of the study, a symptom which completely disappeared after 5 days. However, when relatively large doses (9-13 g/day) of glucomannan were supplemented by mixing into biscuits, subjects experienced transient flatulence and soft stools (Vuksan *et al.* 1999, Vuksan *et al.* 2000), indicating that supplementation of a larger amount of

glucomannan may induce negative gastrointestinal symptoms. For example, Marsicano *et al.* (1995) evaluated the effect of 2 doses of glucomannan (3 g/day and 4 g/day) for 5 weeks on the intestinal habits and stool characteristics in 50 patients. An increase in the number of daily and weekly evacuations was observed in the glucomannan treated group compared to the placebo group. Bowel evacuation during the 3 g/day glucomannan phase increased 0.5 times per day or 3 more per week. On the 4 g/day treatment of glucomannan evacuation increased 0.9 times per day, or 6 more per week. Therefore, a small increase in the dose of glucomannan may have strong effects on intestinal movements.

RATIONALE

Dyslipidemia often occurs in type 2 diabetic individuals and cholesterol metabolism is altered in these patients, possibly due to decreased insulin sensitivity and disturbed glucose metabolism. Elevated circulating cholesterol frequently occurs in type 2 diabetic patients. Since hypercholesterolemia increases risk for developing CVD, it is important to reduce circulating cholesterol concentrations for type 2 diabetic and non-diabetic individuals at a risk. Plant sterols lower circulating cholesterol levels by reducing intestinal cholesterol absorption. However, this cholesterol-lowering efficacy is often partially compensated for by a simultaneous increase in cholesterol synthesis. A soluble fiber, glucomannan, lowers cholesterol levels by decreasing cholesterol synthesis, by decreasing the rate of digestion and postprandial insulin secretion which reduce HMG-CoA reductase activity. Glucomannan is also reported to improve glycemic control. In light of the fact that plant sterols and glucomannan decrease circulating cholesterol levels through different mechanisms, it is important to assess whether the combination of plant sterols and glucomannan has a synergistic or additive hypocholesterolemic effect in type 2 diabetic and non-diabetic subjects.

Thus, the purpose of the present study was to examine the effects of plant sterols and glucomannan alone and in combination of both, on cholesterol absorption, synthesis, and plasma lipid levels as well as glycemic control in mildly hypercholesterolemic type 2 diabetic and non-diabetic subjects.

HYPOTHESES

(1) Ho: Circulating lipid levels, non-cholesterol sterol profiles as well as glycemic control in response to supplementation of 1) plant sterols, 2) glucomannan, 3) a combination of each, and 4) placebo, will not differ in mildly hypercholesterolemic type 2 diabetic and non-diabetic individuals.

(2) Ho: There will be no differences between type 2 diabetic and non-diabetic individuals in circulating lipid levels, non-cholesterol sterol profiles, and glycemic controls in response to the consumption of plant sterols and/or glucomannan

(3) Ho: There will be no interactive effect on circulating lipid levels, non-cholesterol sterol profile, and glycemic controls between two subject groups (type 2 diabetic and non-diabetic group) and supplementation of plant sterols and/or glucomannan.

OBJECTIVES

(1) To characterize the effects of supplementation of plant sterols and/or glucomannan on circulating lipid levels, non-cholesterol sterol profiles as well as glycemic control in type 2 diabetic and in non-diabetic individuals without dietary controls.

(2) To determine if changes in plasma lipid concentrations, non-cholesterol sterol profiles and glycemic control by supplementation of plant sterols and/or glucomannan are different between type 2 diabetic and non-diabetic individuals.

(3) To determine if an interaction exists between changes in plasma lipid concentrations, non-cholesterol sterol profile and glycemic control between type 2 diabetic and non-diabetic groups and supplementation of plant sterols and/or glucomannan.

CHAPTER 3. MANUSCRIPT

ADDITIVE EFFECT OF PLANT STEROLS AND GLUCOMANNAN IN LOWERING LOW-DENSITY LIPOPROTEIN CHOLESTEROL IN INDIVIDUALS WITH AND WITHOUT TYPE 2 DIABETES

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3.1. Abstract

Background: Dyslipidemia accelerates the development of cardiovascular disease (CVD) and is a common complication in type 2 diabetic individuals. Although it is known that plant sterols and a viscous soluble-fiber, glucomannan have cholesterol-lowering effects when used alone, few studies have investigated their combined effects.

Objectives: To assess 1) the effects of glucomannan and plant sterols on lipid profile, glycemic control as well as plasma plant sterol concentrations in mildly hypercholesterolemic type 2 diabetic and non-diabetic subjects, and 2) whether the response of type 2 diabetic and non-diabetic groups to the treatments is similar.

Methods: Thirteen type 2 diabetic and sixteen non-diabetic subjects participated in a randomized crossover trial that consisted of 4 phases of 21 days, each phase separated by a 28-day washout. Subjects were supplemented with either 1) plant sterols (1.8 g/day), 2) glucomannan (10 g/day), 3) a combination of glucomannan and plant sterols, or 4) placebo, provided in the form of granola bars.

Results: Plasma total cholesterol levels decreased ($p<0.05$) from baseline by -7.8%, -12.4%, -14.8%, and -3.2 % with plant sterol, glucomannan, combination and control treatments, respectively. Plasma low-density lipoprotein (LDL) cholesterol levels were decreased ($p<0.05$) by -10.6%, -14.5%, -21.3% and -4.5% in the plant sterol, glucomannan, combination and control treatment groups, respectively. Plasma triglyceride and high-density lipoprotein (HDL) cholesterol levels as well as glycemic control did not change across treatments. Plasma lathosterol levels, an index of cholesterol biosynthesis, were decreased ($p<0.05$) after the combination treatment.

Overall plasma campesterol levels were lower ($p<0.05$) in the type 2 diabetic group compared to the non-diabetic group during the study period.

Conclusion: The results suggest that although supplementation of plant sterols and glucomannan did not change plasma insulin, serum fructosamine and blood glucose levels during a 2hr oral glucose tolerance test, a combination of glucomannan and plant sterols substantially improved plasma lipids likely by reducing cholesterol absorption and synthesis simultaneously. Supplementation with plant sterols and glucomannan can be considered an alternative approach to manage circulating cholesterol levels in both type 2 and non-diabetic individuals.

3.2. Introduction

Diabetes mellitus is a common disease that afflicts people worldwide. It has been reported that type 2 diabetes accounts for about 90% of all diabetic patients (Anderson, 1998). Genetic and environmental factors have been attributed to type 2 diabetes (Polonsky *et al.* 1996). Type 2 diabetes is characterized by insulin resistance and impaired β-cell function in the pancreas. The reduced insulin secretion and/or insulin sensitivity results in elevated blood glucose concentrations, which in turn deteriorates organs, in particular blood vessels and nerves. It has been reported that type 2 diabetic patients have a 3-fold higher risk of coronary artery disease compared with non-diabetic individuals, (Hsueh and Law, 1998) as well as higher risk of cardiovascular morbidity and mortality (Garcia *et al.* 1974). It is evident that a hyperglycemic condition affects lipid metabolism, and dyslipidemia occurs frequently in type 2 diabetic individuals (Reaven 1988, Feingold *et al.* 1992). Some investigators also report that altered cholesterol metabolism exists among type 2 diabetic individuals (Briones *et al.* 1986, Gylling and Miettinen, 1997). The most common dyslipidemic symptoms of type 2 diabetes are characterized by hypertriglyceridemia, decreased concentrations of high-density lipoprotein (HDL) cholesterol, and reduced size of low-density lipoprotein (LDL) particles (Austin and Edwards, 1996, Pascot *et al.* 2001). Elevated LDL cholesterol levels are also frequently found in patients with type 2 diabetes (Kannel and McGee, 1979). Since increased LDL cholesterol concentrations are risk factor of developing CVD (Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults, 2001), it is important to maintain the optimal circulating cholesterol levels as well as glucose concentrations in

type 2 diabetes (Hsueh and Law, 1998) in order to prevent further complications such as CVD.

Plant sterols have been investigated as cholesterol-lowering agents since the early 1950's (Pollack, 1953). The efficacy of plant sterols has now been unquestionably established through many earlier (Weststrate and Meijer, 1998) and recent studies (Hallikainen *et al.* 2000a, Jones *et al.* 2000, Vanstone *et al.* 2002) and often used as supplements by type 2 diabetic (Gylling and Miettinen,1996, Lee *et al.* 2003) and non-diabetic adults, (Weststrate and Meijer, 1998, Hallikainen *et al.* 2000a. Miettinen *et al.* 2000, Clifton, 2002, Vanstone *et al.* 2002) as well as familial hypercholesterolemic children (Becker *et al.* 1993, Gylling *et al.* 1995, Amundsen *et al.* 2002). As a proposed mechanism for the cholesterol-lowering action by plant sterols, it has been shown that plant sterols reduce intestinal cholesterol absorption by competing with cholesterol to form micelles (Ikeda *et al.* 1989, Plat and Mensink, 1999). Recently, it was observed that plant stanols also reduce cholesterol absorption by increasing ATP-Binding Cassette transporter A1 expression thereby enhancing cholesterol efflux back into the intestinal lumen from enterocytes (Plat and Mensink, 2002b).

As a viscous soluble fiber, glucomannan has been acknowledged as a cholesterol-lowering (Doi *et al.* 1979, Haskell *et al.* 1992, Vuksan *et al.* 2000) and hypoglycemic agent (Doi *et al.* 1979, Scalfi *et al.* 1987, Hopman *et al.* 1988, Huang *et al.* 1990, Vuksan *et al.* 2000, McCarty, 2002, Chen *et al.* 2003). In a liquid, glucomannan increases in volume and becomes a viscous gel (Doi, 1995). This rheological property of glucomannan is an important determinant for its hypocholesterolemic and hypoglycemic

abilities (Arvill and Bodin, 1995, Vuksan *et al.* 2000, McCarty, 2002). Glucomannan reduces the postprandial plasma glucose and insulin concentrations (Jenkins *et al.* 1978, Doi *et al.* 1979, Kim *et al.* 1996) by slowing down the rate of carbohydrate absorption (Doi, 1995). This reduction of postprandial insulin concentration is thought to decrease the endogenous cholesterol synthesis (Jones *et al.* 1993), by reducing insulin-induced 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity (Rodwell *et al.* 1976). It has been also hypothesized that glucomannan lowers cholesterol levels by increasing hepatic bile acid production and fecal excretion of neutral sterols and bile acids (Vuksan *et al.* 2000).

Since plant sterols and glucomannan decrease the circulating cholesterol levels through different mechanisms, it is important to assess whether they act in a synergistic or additive hypocholesterolemic manner in type 2 diabetic and non-diabetic subjects. Thus, the primary objective of this study was to examine the effects of plant sterols and glucomannan alone and in combination on cholesterol absorption, synthesis, and plasma lipid levels, as well as glycemic control in type 2 diabetic and non-diabetic subjects. The secondary objective was to determine if the responses to supplementations of plant sterols and/or glucomannan were different between type 2 diabetic and non-diabetic individuals.

3.3. Experimental design and methods

Subjects

Sixteen non-diabetic (7 men and 9 women) and 13 type 2 diabetic (4 men and 9 women) volunteers with mild hypercholesterolemia were recruited through advertisements in local newspapers. Subjects were aged between 38 and 74 years. Female subjects were postmenopausal. Subjects were asked to provide a medical history and to complete a physical examination prior to acceptance into the study. Fasting blood and urine samples were collected screened for biochemical, hematological, and urine indices. Subjects with serum LDL cholesterol levels between 2.8 and 6.9 mmol/L and triglyceride levels < 4 mmol/L were selected for the study. Based on the diagnostic criteria of American Diabetic Association, diabetic subjects were required to have fasting blood glucose levels > 7 mmol/L and HbA1c levels between 6 and 9%. For non-diabetic subjects, the criteria for fasting blood glucose levels was < 6.1 mmol/L. Subjects who were receiving hypolipidemic or insulin therapy, or suffered from chronic diseases such as gastrointestinal, renal, pulmonary, hepatic or biliary disease, or had a history of angina, congestive heart failure, or chronic use of laxatives were excluded from the study. Subjects were permitted to continue their medication of metformin, sulfonylurea, thyroid hormone, antihypertensive agents, and postmenopausal estrogens during the entire study.

Experimental Design and Protocol

The study was a randomized crossover clinical trial involving 4 treatment phases of 21 days each and separated by a 4 week washout period between phases. Subjects were assigned to all 4 treatments in a random order and consumed 1.8 g/day of plant sterols, 10

g/day of glucomannan, a combination of both, or none as a control. The plant sterol mixture contained 67.3% sitosterol, 10.8% sitostanol, 8.2% campesterol, 1.6% campestanol, and 12.1% of other phytosterols. Plant sterols and glucomannan were provided in the form of granola bars (Forbes Medi-tech Inc. Vancouver, BC, Canada). Nutritional compositions of the granola bars are presented in **Table 3.1.** Subjects were instructed to take 1 bar between meals as a snack, 3 times a day together with 250 mL of any type of beverage. The bars were provided to subjects each week (day 0, 8, and 15) at the Mary Emily Clinical Nutrition Research Unit of McGill University (Montreal, QC, Canada). Compliance was assessed by asking all research subjects to return uneaten portions of bars provided, then weighing the amount of returned bars weekly. Palatability of the treatment bars was evaluated on a scale from 0 to 10 at the end (day 21) of each phase. A higher score indicated greater palatability. Physical examinations were performed at the beginning (day 0) and the end (day 21) of each phase by the study physician to ensure the health status of all participants. During the study subjects were instructed to replace their carbohydrate source with treatment bars partially and to maintain the other usual dietary habits. Body weight was measured on days 0, 8, 15, and 21. Side effects and dietary changes during the study period were monitored by questionnaire at the beginning (day 0) and the end (day 21) of each treatment phase.

Fasting blood samples were taken at day 0, 8, 15, and 21, during each phase. Plasma and red blood cell were separated by centrifugation for 15 min at 1500 rpm within 30 min of phlebotomy and were stored at –20 °C until analysis. Plant sterols, insulin, and fructosamine levels were measured at the beginning (day 0) and end of each phase (day 21). On the last day of each phase (day 21), after fasting blood sample collection, a 2-

hour oral glucose tolerance test was conducted before breakfast. A liquid containing 50 g glucose was given orally to each subject and finger prick blood samples were taken every half hour for 2 hours. The palatability of the treatment granola bars was evaluated by questionnaire at the end of each treatment phase.

All subjects gave informed consent. Prior to participating in the study subjects were given the opportunity to discuss any queries with the primary investigator, the physician, or the study coordinator. Ethical approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine at McGill University.

Determination of plasma lipid concentrations

Plasma total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride concentrations were determined enzymatically using a Hitachi/991 chemistry analyzer (Roche Diagnostic Inc. Indiana IN) at the Lachine Hospital (Montreal, QC, Canada) (Siedel *et al.* 1983, Sugiuchi *et al.* 1995). Plasma LDL cholesterol levels were calculated using the Friedewald equation (Friedewald *et al.* 1972).

Determination of plasma non-cholesterol sterol concentrations

Plasma plant sterol concentrations were measured in duplicate by gas-liquid chromatography as described elsewhere (Ntanios and Jones, 1998). Briefly, 1.0 mL of plasma was saponified with methanolic KOH at 100 °C for 2 h. The non-saponified lipids were extracted with petroleum ether. An internal standard of 5-α-cholestane was added at the beginning of lipid extraction. The lipid extracts were analyzed by a gas-liquid chromatography equipped with a flame ionization detector (HP 5890 series II; Hewlett

Packard, Palo Alto CA) using a 30 m capillary column with i.d. of 0.25 mm (SAC-5; Supelco, Bellefonte PA). Detector and injector temperatures were set at 310 and 300 °C, respectively. Samples were run isothermally at 285 °C. Plant sterol peaks were identified by comparison with authenticated standards (Sigma-Aldrich Canada Ltd., Oakville ON, Canada).

Determination of plasma insulin, serum fructosamine and blood glucose concentrations

Plasma insulin concentrations were determined in duplicate, using commercially available radioimmunoassay kits (ICN Pharmaceutical, Inc., Costa Mesa, CA) utilizing ^{125}I as a tracer. Radioactivity was determined by gamma counter (LKB Wallac, 1282 compugamma CS, Fisher Scientific, Montreal, QC, Canada) and expressed as count per min (CPM). Plasma insulin concentrations were quantified in reference to a standard curve. Serum fructosamine concentrations were determined by LDS Diagnostic Laboratories (Pointe Claire, QC, Canada). Blood glucose levels were measured using a portable glucometer, Glucometer Elite® XL (Bayer Inc., Toronto, ON, Canada).

Statistics

Data were analyzed by repeated measures analysis of variance using general linear models procedure (version 6.12; SAS Institute Inc, Cary, NC). Normal distribution of all variables was examined using Shapiro-Wilk test for normality. When treatment effects were significant, comparisons between individual treatments were conducted using

Scheffé post-hoc test to identify the significant effects of each treatment. The p value < 0.05 was considered statistically significant. All data are presented as the mean ± S.E.M.

3.4. Results

Characteristics of subjects

Sixteen type 2 diabetic and 18 non-diabetic individuals participated the study, with 13 diabetic and 16 non-diabetic subjects completing all treatments. Two subjects (1 non-diabetic and 1 diabetic subject) withdrew their participation after glucomannan supplementation, due to gastric discomfort. Results of 3 subjects (1 non-diabetic and 2 diabetic subjects) were excluded from the statistical analysis due to poor compliance in supplement intake (< 50%), which was evaluated by the weight of the returned bars. The baseline characteristics of subjects are presented in **Table 3.2.** Body mass index (BMI), plasma triglyceride, and all of the diabetic indicators including glucose, insulin, HbA1c and fructosamine were higher ($p<0.05$) in the type 2 diabetic group than the non-diabetic group. Plasma LDL cholesterol levels were lower ($p<0.01$) in diabetic subjects compared with non-diabetic individuals. However, because there is a higher risk of developing CVD in type 2 diabetes, both of these groups are characterized as mildly hypercholesterolemic. Decreased HDL cholesterol and elevated triglyceride concentrations were found in type 2 diabetic group, which are commonly seen dyslipidemic profiles in type 2 diabetes. HbA1c (0.07 ± 0.003) was relatively low in the diabetic group, implying good glycemic control at baseline in the type 2 diabetic subjects.

The oral hypoglycemic agents administered included metformin (5 subjects), combination of metformin and glyburide (6 subjects), and combination of glyburide and losartan (1 subject). None of the participants received insulin therapy and hypolipidemic medication.

Compliance

Based on the weight of returned bars, and excluding subjects dropped from the study due to poor compliance, consumption of test granola bars was calculated to be 98.8%, 99.4%, 98.4%, and 98.8% in control, plant sterols, glucomannan, and combination treatments, respectively. There was no difference between the two groups of subjects or between dietary treatments in the consumption of granola bars.

Palatability of treatment granola bars

The palatability of the granola bars was rated at 7.16 ± 0.39, 7.63 ± 0.38, 6.36 ± 0.48, and 6.04 ± 0.36 in the control, plant sterols, glucomannan, and combination treatments, respectively. Plant sterol-containing granola bars showed higher ($p<0.05$) palatability than glucomannan-containing bars or the bars containing both glucomannan and plant sterols. Type 2 diabetic subjects showed higher acceptance for the tested granola bars than non-diabetics.

Intestinal changes in response to treatment

The number of bowel evacuations increased in 8, 12, and 14 subjects during plant sterols, glucomannan, and the combination treatments, respectively. Soft stool was observed in 8, 14, 12 subjects during plant sterols, glucomannan, and the combination treatments, respectively. During the control phase, 12 subjects reported that their stools became harder. Twenty-one subjects reported increased gas production during treatments containing glucomannan. However, the majority of the participants reported that the changes in the intestinal habits and stool characteristics were tolerable.

Effects of dietary treatments on body weight

Mean body weight changes from baseline were -0.22 ± 0.32, -0.10 ± 0.18, -0.05 ± 0.18, and -0.70 ± 0.24 kg in control, plant sterols, glucomannan, and the combination treatments, respectively. During the combination treatment, type 2 diabetic subjects decreased ($p<0.05$) their body weight (-1.34 ± 0.24 kg), while non-diabetic subjects maintained their body weight (-0.29 ± 0.33 kg). There was no correlation between changes in weight and other parameters (data not shown). In other treatment periods, no significant changes in body weight were observed as compared to baseline or between the two groups of subjects. No significant changes in dietary habit and medication were reported during the entire study period.

Effects of dietary treatments on plasma lipids

Plasma lipid levels of non-diabetic and type 2 diabetic groups during the treatment periods are shown in **Table 3.3.** Supplementation of glucomannan combined with plant sterols reduced ($p<0.05$) plasma total cholesterol levels (4.77 ± 0.20 mmol/L) compared to control (5.38 ± 0.18 mmol/L). The percentage change of total cholesterol from baseline was lower ($p<0.05$) in glucomannan (-12.40 ± 2.32%) and combination (-14.80 ± 3.86%) treatments compared to control (-3.20 ± 1.49%). There was no difference between groups of subjects in response to the treatments. The weekly changes of plasma total cholesterol levels are shown in **Figure 3.1.** After 1 week, plasma total cholesterol levels were lower ($p<0.05$) in the plant sterol, glucomannan, and combination treatments compared to control. Although the degree of reduction of total cholesterol was gradually diminished after 2 weeks of plant sterol and glucomannan supplementation, the decrease in total cholesterol concentrations was still observed with the combination treatment.

On day 0 of the glucomannan treatment, plasma LDL cholesterol levels were lower ($p<0.05$) in the type 2 diabetic group (3.47 ±0.17 mmol/L) compared with the non-diabetic group (4.06 ± 0.20 mmol/L). After 21 days of supplementation, glucomannan (3.18 ± 0.14 mmol/L) and the combination of glucomannan and plant sterols (3.00 ± 0.16 mmol/L) decreased ($p<0.05$) LDL cholesterol levels compared to control (3.53 ± 0.16 mmol/L). Plasma LDL cholesterol levels were also lower ($p<0.05$) after combination treatment (3.00 ± 0.16 mmol/L) compared with plant sterol treatment (3.43 ± 0.13 mmol/L). The weekly changes of LDL cholesterol levels during the study are shown in **Figure 3.2.** LDL cholesterol was reduced ($p<0.05$) after 1 week of glucomannan and combination treatment. LDL cholesterol levels were reduced ($p<0.05$) by -21.27± 3.76%, with the combination treatment from day 1 to day 21 compared with -4.51 ± 3.02% in the control. There was no difference between the two groups of subjects in the changes of LDL cholesterol levels after each dietary treatment

Plasma HDL cholesterol levels did not differ among the treatment period. There was also no difference in the changes of HDL levels between two subject groups.

The ratio of plasma LDL cholesterol to HDL cholesterol was lower ($p<0.05$) after plant sterol (3.41 ± 0.19) and glucomannan treatments (3.27 ± 0.17) compared to control (3.68 ± 0.19). Furthermore, plasma LDL cholesterol to HDL cholesterol ratio in the combination treatment (3.11 ± 0.17) was lower ($p<0.05$) than both the control (3.68 ± 0.19) and plant sterol (3.41 ± 0.19) treatments. No differences were observed between the two groups in the LDL to HDL cholesterol ratio.

Total cholesterol to HDL cholesterol ratio was lower ($p<0.05$) after 21 days on the combination treatment (5.03 ± 0.22) compared to control (5.65 ± 0.25) and plant sterol treatments (5.36 ± 0.28). Total cholesterol to HDL cholesterol ratio was lower ($p<0.05$) in subjects taking glucomannan (5.20 ± 0.24) compared to control treatment (5.65 ± 0.25).

As shown in **Table 3.3.**, plasma triglyceride levels were not affected by dietary treatments. However, the plasma triglyceride levels were consistently higher ($p<0.05$) in diabetic group (2.14 ± 0.11 mmol/L) than in non-diabetic group (1.32 ± 0.08 mmol/L) during the entire study period.

Effects of dietary treatments on plasma non-cholesterol sterols

Plasma plant sterols in type 2 diabetic and non-diabetic groups are summarized in **Table 3.4.** The overall mean of plasma campesterol levels was lower ($p<0.05$) in the diabetic group (5.77 ± 0.42 μmol/L) than in the non-diabetic group (7.59 ± 0.38 μmol/L). There was no difference among treatments.

On day 21, β-sitosterol levels and the ratio of β-sitosterol to total cholesterol were higher ($p<0.05$) following plant sterol treatment (5.50 ± 0.76 μmol/L and 1.11 ± 0.18 mmol/mol) compared to the glucomannan treatment (3.81 ± 0.45 μmol/L and 0.75 ± 0.45 mmol/mol). Plasma β-sitosterol levels were not different between type 2 diabetic and non-diabetic groups after each treatment.

There was no difference in the plasma lathosterol levels between the two groups in response to the treatments. However, the reductions after 21 days were greater ($p<0.05$) with the combination treatment (-1.25 ± 0.36 μmol/L) compared with plant sterol treatment (0.17 ± 0.40 μmol/L).

Effects of dietary treatments on insulin, fructosamine, and 2hr oral glucose tolerance test

Overall plasma fasting insulin levels were higher ($p<0.01$) in the diabetic group (280.97 ± 16.07 pmol/L) compared to the non-diabetic group (150.75 ± 7.18 pmol/L). Overall serum fructosamine levels were also higher ($p<0.01$) in diabetic subjects (3.95 ± 0.07 umol/g) compared to non-diabetic subjects (3.40 ± 0.03 umol/g). Blood glucose levels were consistently higher ($p<0.01$) in type 2 diabetic than in non-diabetic subjects during the 50 g 2 hour oral glucose test. Dietary treatments did not affect plasma insulin and serum fructosamine concentrations, or the results of the glucose tolerance test.

Table 3.1. Nutritional composition of tested bars across treatments

Nutrients (per day)	Control	Plant sterols	Glucomannan	Combination
Total energy (kcal)	362.7	387.9	327.3	355.2
Carbohydrate (g)	60.6	59.4	53.1	51.3
Protein (g)	7.5	7.2	5.7	6.0
Fat (g)	10.2	13.5	10.2	14.1
Saturated fat (g)	1.8	1.2	2.1	1.2
Sodium (mg)	280.5	190.2	249.9	158.4
Fiber (g)	4.5	4.5	13.5	13.5
Vitamin A (IU)	775.2	437.7	698.7	363.0
Vitamin C (mg)	4.8	4.8	3.6	3.9
Fe (mg)	2.4	2.4	2.1	2.1
Calcium (mg)	43.8	40.2	38.7	35.1

Table 3.2. Baseline characteristics of subjects[1]

Variables	Non-diabetic (n=16)	Type 2 diabetic (n=13)
Male/Female	7/9	4/9
Ages (y)	55.2 ± 1.7	56.8 ± 3.1
Body mass index (kg/m^2)	27.7 ± 1.1	31.0 ± 1.0*
Lipids (mmol/L)		
Total cholesterol	6.1 ± 0.2	5.5 ± 0.2
LDL cholesterol[2]	4.3 ± 0.2	3.5 ± 0.2**
HDL cholesterol[3]	1.1 ± 0.1	0.9 ± 0.1
Triacylglycerol	1.4 ± 0.1	2.7 ± 0.4**
Glucose (mmol/L)	5.1 ± 0.1	8.6 ± 0.4***
Insulin (pmol/L)	21.0 ± 2.0	36.0 ± 4.50**
HbA1c (%)	5.5 ± 0.1	7.0 ± 0.3***
Fructosamine (umol/L)	3.5 ± 0.1	4.1 ± 0.2***

[1]Values are expressed as mean ± S.E.M. * P<0.05, ** P<0.01, *** P<0.001 significant difference from non diabetic group.
[2]Low-density lipoprotein cholesterol
[3]High-density lipoprotein cholesterol

Table 3.3. Plasma lipid levels at day 0 and day 21 of each treatment period [1]

Lipid (mmo/L)	Control	Plant sterols	Glucomannan	Combination
Total cholesterol				
Non-diabetics				
Day 0	5.88 ± 0.21	5.90 ± 0.21	5.86 ± 0.23	5.81 ± 0.29
Day 21	5.52 ± 0.28	5.46 ± 0.19	5.07 ± 0.27	4.77 ± 0.35
% Change	-1.82 ± 1.95	-7.31 ± 1.75	-13.92 ± 3.23	-16.79 ± 6.67
% Relative to C[2]		-4.36 ± 1.84	-12.57 ± 2.85	-18.23 ± 5.10
Type 2 diabetics				
Day 0	5.61 ± 0.18	5.54 ± 0.19	5.42 ± 0.19	5.49 ± 0.20
Day 21	5.21 ± 0.20	5.06 ± 0.23	4.84 ± 0.16	4.77 ± 0.15
% Change	-4.93 ± 2.30	-8.41 ± 2.12	-10.50 ± 3.38	-12.3 ± 2.76
% Relative to C[2]		-2.21 ± 3.96	-5.95 ± 4.11	-7.71 ± 2.81
All				
Day 0	5.76 ± 0.14	5.74 ± 0.14	5.66 ± 0.16	5.67 ± 0.18
Day 21	5.38 ± 0.18 [a]	5.28 ± 0.15 [ab]	4.97 ± 0.16 [ab]	4.77 ± 0.20 [b]
% Change	-3.20 ± 1.49 [a]	-7.80 ± 1.33 [ab]	-12.40 ± 2.32 [b]	-14.80 ± 3.86 [b]
% Relative to C[2]		-3.36 ± 2.05 [a]	-9.50 ± 2.48 [ab]	-13.35 ± 3.14 [b]
LDL cholesterol[3]				
Non-diabetics				
Day 0	4.08 ± 0.20	4.10 ± 0.21	4.06 ± 0.20	4.05 ± 0.24
Day 21	3.77 ± 0.24	3.66 ± 0.15	3.42 ± 0.23	3.11 ± 0.26
% Change	-3.07 ± 3.31	-10.67 ± 2.14	-15.78 ± 4.04	-26.38 ± 6.00
% Relative to C[2]		-5.42 ± 2.28	-14.33 ± 3.44	-22.73 ± 5.42
Type 2 diabetics				
Day 0	3.61 ± 0.22	3.60 ± 0.15	3.47 ±0.17*	3.51 ± 0.18
Day 21	3.26 ± 0.17	3.17 ± 0.20	2.90 ± 0.12	2.81 ± 0.15
% Change	-6.21 ± 5.44	-10.45 ± 4.11	-12.92 ± 4.06	-15.24 ± 3.87
% Relative to C[2]		-1.56 ± 5.40	-8.42 ± 5.63	-12.84 ± 3.71
All				
Day 0	3.88 ± 0.15	3.89 ± 0.14	3.81 ± 0.14	3.82 ± 0.17
Day 21	3.53 ± 0.16 [a]	3.43 ± 0.13 [ab]	3.18 ± 0.14 [bc]	3.00 ± 0.16 [c]
% Change	-4.51 ± 3.02 [a]	-10.57 ± 2.16[ab]	-14.47 ± 2.83 [ab]	-21.27 ± 3.76 [b]
% Relative to C[2]		-3.56 ± 2.82 [a]	-11.59 ± 3.19 [b]	-18.14 ± 3.45 [b]

Lipid (mmol/L)	Control	Plant sterols	Glucomannan	Combination
Triacylglycerol				
Non-diabetics				
Day 0	1.42 ± 0.15	1.42 ± 0.14	1.35 ± 0.13	1.29 ± 0.12
Day 21	1.41 ± 0.16	1.40 ± 0.15	1.19 ± 0.15	1.29 ± 0.19
% Change	-1.80 ± 6.91	5.44 ± 8.77	-14.28 ± 6.37	-6.47 ± 6.65
% Relative to C[2]		5.57 ± 8.64	-11.01 ± 8.47	-5.32 ± 11.94
Type 2 diabetics				
Day 0	2.32 ± 0.29**	2.16 ± 0.23**	2.03 ± 0.19**	2.20 ± 0.19***
Day 21	2.22 ± 0.24**	2.00 ± 0.23*	2.15 ± 0.25**	2.18 ± 0.22**
% Change	0.95 ± 13.14	-10.54 ± 7.63	-0.25 ± 10.23	-3.22 ± 7.47
% Relative to C[2]		-3.00 ± 10.56	5.07 ± 11.41	6.55 ± 10.27
All				
Day 0	1.82 ± 0.17	1.75 ± 0.14	1.65 ± 0.13	1.70 ± 0.14
Day 21	1.78 ± 0.16	1.68 ± 0.14	1.63 ± 0.17	1.71 ± 0.16
% Change	-0.48 ± 7.13	-2.26 ± 5.94	-7.53 ± 5.97	-4.90 ± 4.90
% Relative to C[2]		1.64 ± 6.66	-3.55 ± 7.01	0.19 ± 7.91
HDL cholesterol[4]				
Non-diabetics				
Day 0	1.11 ± 0.09	1.13 ± 0.09	1.07 ± 0.08	1.09 ± 0.09
Day 21	1.03 ± 0.07	1.11 ± 0.09	1.05 ± 0.09	0.99 ± 0.08
% Change	-0.79 ± 2.62	-2.95 ± 2.33	-3.63 ± 3.02	-5.20 ± 7.06
% Relative to C[2]		8.89 ± 8.99	3.63 ± 9.39	0.11 ± 9.92
Type 2 diabetics				
Day 0	0.97 ± 0.05	0.97 ± 0.06	0.99 ± 0.06	0.96 ± 0.04
Day 21	0.94 ± 0.06	0.99 ± 0.06	0.96 ± 0.06	0.96 ± 0.05
% Change	-3.28 ± 3.72	2.81 ± 2.40	-2.56 ± 2.69	0.55 ± 1.65
% Relative to C[2]		6.00 ± 3.51	2.97 ± 3.82	4.30 ± 3.42
All				
Day 0	1.05 ± 0.05	1.05 ± 0.06	1.03 ± 0.05	1.03 ± 0.55
Day 21	0.99 ± 0.04	1.05 ± 0.06	1.01 ± 0.05	0.98 ± 0.05
% Change	-1.94 ± 2.20	-0.27 ± 1.73	-3.34 ± 2.01	-2.53 ± 3.84
% Relative to C[2]		7.59 ± 5.13	3.33 ± 5.37	1.99 ± 5.61

Lipid (mmol/L)	Control	Plant sterols	Glucomannan	Combination
LDL:HDL ratio[3,4]				
Non-diabetics				
Day 0	4.03 ± 0.36	3.97 ± 0.37	4.21 ± 0.36	3.69 ± 0.41
Day 21	3.78 ± 0.32	3.51 ± 0.31	3.40 ± 0.28	3.24 ± 0.28
% Change	-1.14 ± 1.38	0.42 ± 0.85	0.61 ± 1.76	-3.41 ± 2.52
% Relative to C[2]		-6.79 ± 3.33	-8.56 ± 3.60	-14.03 ± 2.92
Type 2 diabetics				
Day 0	3.65 ± 0.25	3.72 ± 0.20	3.50 ± 0.26	3.67 ± 0.23
Day 21	3.56 ± 0.20	3.30 ± 0.23	3.12 ± 0.18	3.00 ± 0.16
% Change	2.38 ± 1.42	-0.71 ± 1.69	2.98 ± 1.20	5.70 ± 3.87
% Relative to C[2]		-7.12 ± 4.13	-11.69 ± 2.92	-16.61 ± 1.76
All				
Day 0	3.87 ± 0.24	3.87 ± 0.23	3.92 ± 0.25	3.68 ± 0.26
Day 21	3.68 ± 0.19 [a]	3.41 ± 0.19 [b]	3.27 ± 0.17 [bc]	3.11 ± 0.17 [c]
% Change	0.30 ± 1.05	-0.43 ± 0.84	1.58 ± 1.16	0.32 ± 1.16
% Relative to C[2]		-6.94 ± 2.57 [a]	-10.01 ± 2.33 [a]	-15.23 ± 1.75 [b]
TC:HDL ratio[4,5]				
Non-diabetics				
Day 0	5.71 ± 0.42	5.71 ± 0.46	5.87 ± 0.43	5.72 ± 0.43
Day 21	5.60 ± 0.40	5.42 ± 0.47	5.20 ± 0.41	5.02 ± 0.37
% Change	-0.95 ± 0.74	0.22 ± 0.67	0.70 ± 1.37	-2.15 ± 1.88
% Relative to C[2]		-3.59 ± 3.30	-6.61 ± 3.43	-10.25 ± 2.30
Type 2 diabetics				
Day 0	5.91 ± 0.30	5.92 ± 0.29	5.70 ± 0.36	5.84 ± 0.27
Day 21	5.71 ± 0.28	5.28 ± 0.28	5.20 ± 0.24	5.04 ± 0.21
% Change	1.75 ± 1.05	-0.76 ± 0.81	1.38 ± 0.88	2.91 ± 2.08
% Relative to C[2]		-7.47 ± 2.78	-8.43 ± 2.67	-11.03 ± 2.51
All				
Day 0	5.80 ± 0.26	5.81 ± 0.28	5.80 ± 0.28	5.77 ± 0.26
Day 21	5.65 ± 0.25 [a]	5.36 ± 0.28 [ab]	5.20 ± 0.24 [bc]	5.03 ± 0.22 [c]
% Change	0.24 ± 0.67	-0.21 ± 0.52	1.00 ± 0.85	0.08 ± 1.46
% Relative to C[2]		-5.33 ± 2.20 [a]	-7.43 ± 2.21 [ab]	-10.60 ± 1.67 [b]

[1]Values are expressed as mean ± S.E.M. Values carrying different superscript letters indicate a significant difference among treatments ($p<0.05$). Percent change is based on individual data. Percent change relative to control is based on the mean of day 21.
*P<0.05; **P<0.01; ***P<0.001; significant difference from non diabetic group.

[2]Control treatment

[3]Low-density lipoprotein cholesterol

[4]High-density lipoprotein cholesterol

[5]Total cholesterol

Table 3.4. Plasma non-cholesterol sterol levels at day 0 and day 21 of each treatment period [1]

Phytosterol (μmol/L)	Control	Plant sterols	Glucomannan	Combination
Campesterol				
Non-diabetics				
Day 21	7.83 ± 0.83	7.35 ± 0.71	7.83 ± 0.91	7.34 ± 0.62
Difference[2]	0.65 ± 0.84	-0.86 ± 0.87	0.79 ± 0.72	-0.33 ± 0.85
mmol/mol[3]	1.48 ± 0.18	1.45 ± 0.21	1.47 ± 0.17	1.66 ± 0.21
Type 2 diabetics				
Day 21	5.59± 0.90	6.46 ± 0.91	5.83 ± 0.85	5.19 ± 0.85*
Difference[2]	-0.51 ± 0.49	0.20 ± 0.89	-0.20 ± 0.90	-0.95 ± 0.96
mmol/mol[3]	1.16 ± 0.24	1.29 ± 0.18	1.18 ± 0.16	1.08 ± 0.15*
All				
Day 21	6.85 ± 0.64	6.96 ± 0.56	6.95 ± 0.65	6.40 ± 0.52
Difference[2]	0.13 ± 0.52	-0.39 ± 0.62	0.35 ± 0.56	-0.61 ± 0.63
mmol/mol[3]	1.34 ± 0.15	1.38 ± 0.14	1.34 ± 0.12	1.41 ± 0.14
B-sitosterol				
Non-diabetics				
Day 21	4.20 ± 0.61	6.08 ± 1.10	3.57 ± 0.51	4.68 ± 0.63
Difference[2]	0.07 ± 0.37	1.02 ± 0.51	-0.31 ± 0.23	0.82 ± 0.39
mmol/mol[3]	0.78 ± 0.11	1.25 ± 0.28	0.69 ± 0.11	1.06 ± 0.18
Type 2 diabetics				
Day 21	4.92 ± 0.76	4.84 ± 1.04	4.09 ± 0.80	4.77 ± 0.85
Difference[2]	0.34 ± 0.42	0.30 ± 0.37	-0.13 ± 0.42	0.30 ± 0.35
mmol/mol[3]	0.99 ± 0.18	0.95 ± 0.20	0.82 ± 0.16	0.99 ± 0.17
All				
Day 21	4.53 ± 0.47 *ab*	5.50 ± 0.76 *a*	3.81 ± 0.45 *b*	4.72 ± 0.51 *ab*
Difference[2]	0.20 ± 0.28	0.68 ± 0.32	-0.22 ± 0.23	0.57 ± 1.32
mmol/mol[3]	0.87 ± 0.10 *ab*	1.11 ± 0.18 *a*	0.75 ± 0.09 *b*	1.03 ± 0.12 *ab*

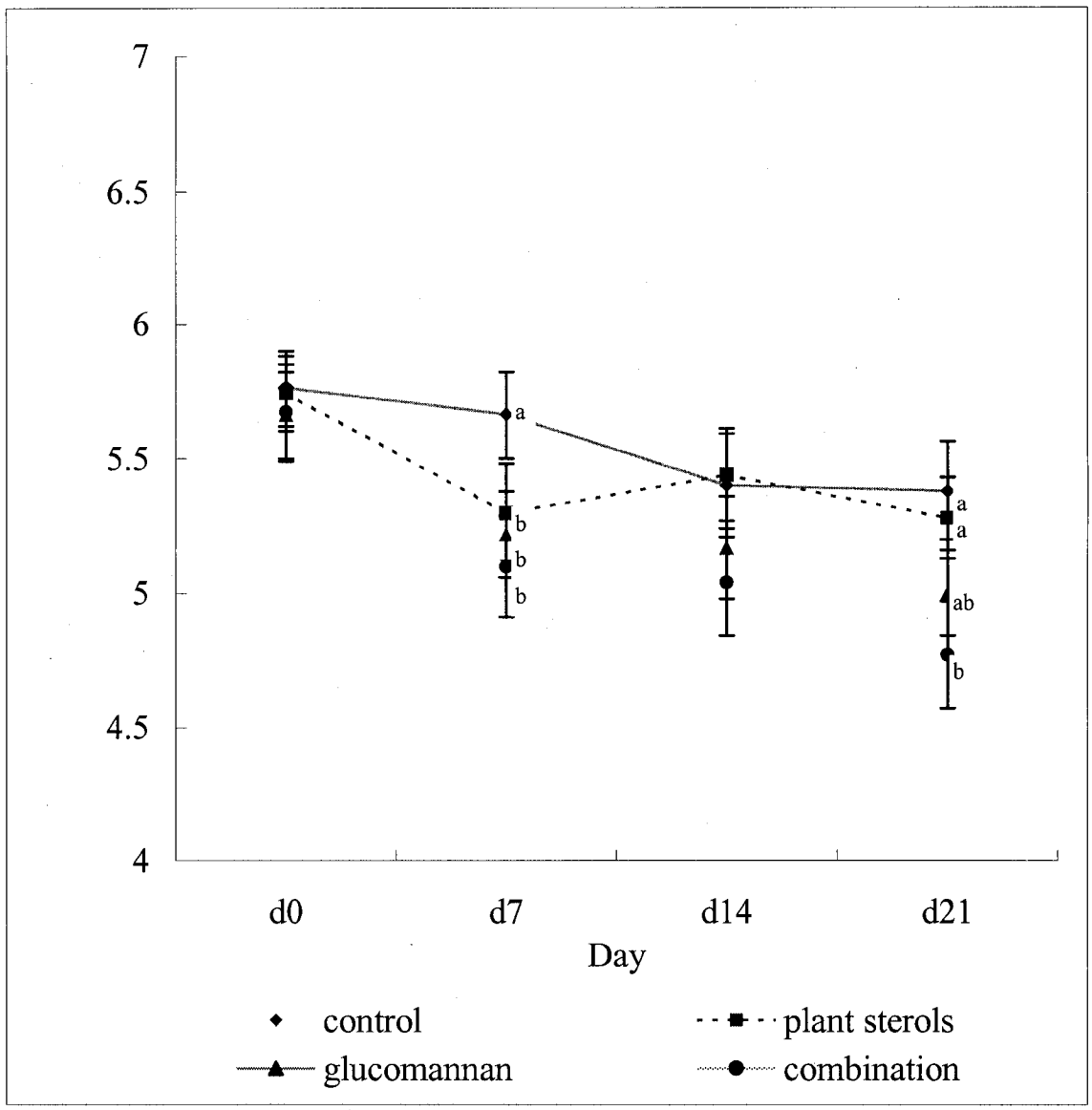
Phytosterol (μmol/L)	Control	Plant sterols	Glucomannan	Combination
Lathosterol				
Non-diabetics				
Day 21	4.65 ± 0.34	4.77 ± 0.52	4.96 ± 0.50	3.86 ± 0.22
Difference[2]	0.47 ± 0.40	0.49 ± 0.57	-1.48 ± 0.53	-1.30 ± 0.27
mmol/mol[3]	0.88 ± 0.08	0.92 ± 0.13	0.94 ± 0.09	0.86 ± 0.09
Type 2 diabetics				
Day 21	4.25 ± 0.58	5.24 ± 0.47	5.03 ± 0.62	4.50 ± 0.28
Difference[2]	-1.25 ± 0.42	0.31 ± 0.59	-0.16 ± 0.51	-1.19 ± 0.72
mmol/mol[3]	0.85 ± 0.13	1.06 ± 0.11	1.03 ± 0.12	0.94 ± 0.05
All				
Day 21	4.47 ± 0.32	4.98 ± 0.35	4.99 ± 0.38	4.15 ± 0.18
Difference[2]	-0.33 ± 0.33[ab]	0.17 ± 0.40 [a]	-0.86 ± 0.38 [ab]	-1.25 ± 0.36 [b]
mmol/mol[3]	0.86 ± 0.07	0.98 ± 0.09	0.98 ± 0.07	0.89 ± 0.06

[1]Values are expressed as mean ± S.E.M. Values carrying different superscript letters indicate a significant difference between groups ($p<0.05$). Percent change is based on individual data. Percent change relative to control is based on the mean of day 21.
*$P<0.05$; significant difference from non diabetic group.

[2]Difference between day 0 and day 21.

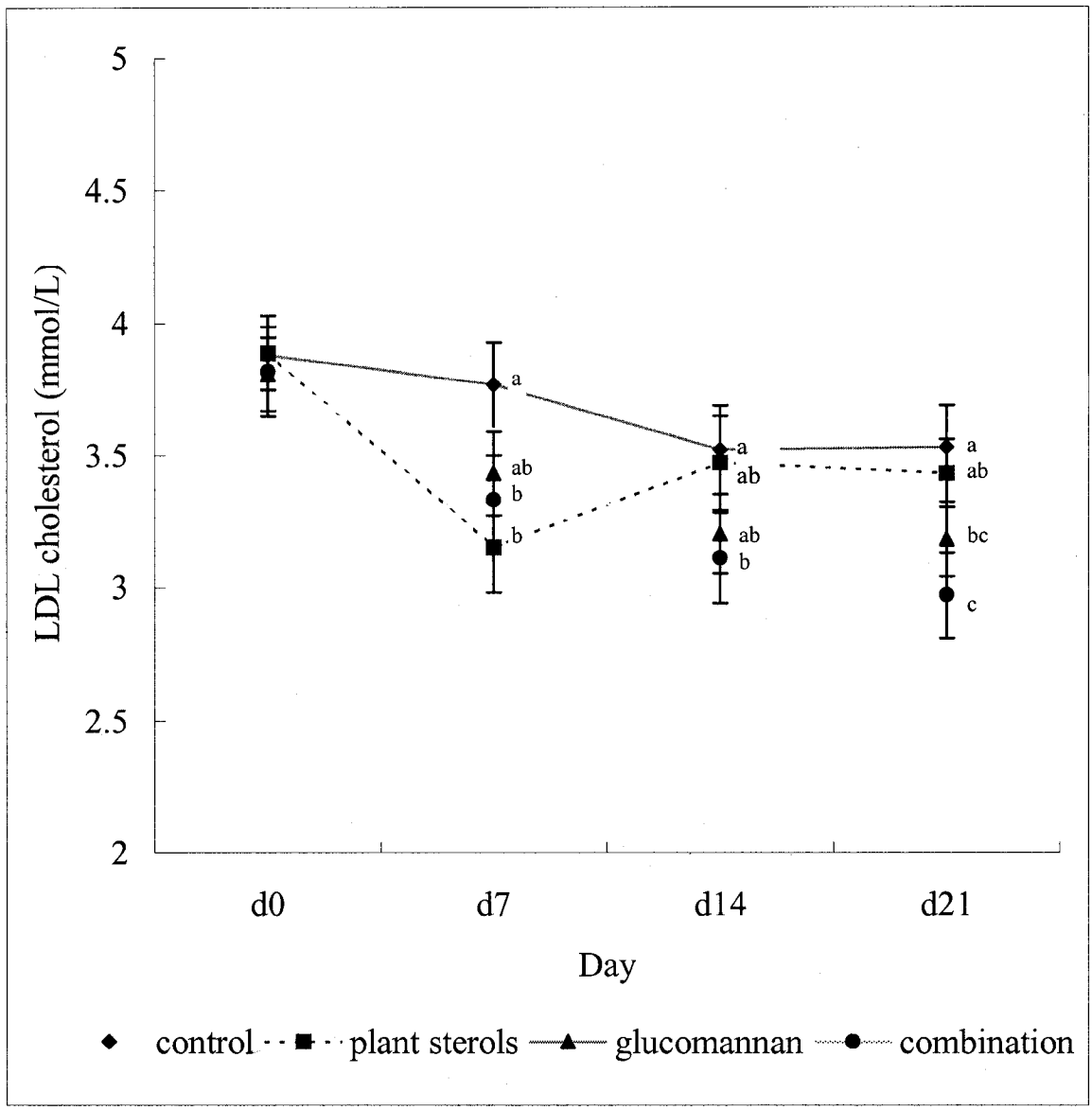
[3]Plant sterol per 1 mol cholesterol.

Figure 3.1. Weekly plasma total cholesterol concentrations across dietary treatments[1]



[1]Values carrying different superscript letters indicate a significant difference between groups ($p<0.05$).
Abbreviation: d - day

Figure 3.2. Weekly plasma low-density lipoprotein cholesterol concentrations across dietary treatments[1]



[1]Values carrying different superscript letters indicate a significant difference between groups ($p<0.05$).
Abbreviation: LDL - low-density lipoprotein cholesterol
d – day

3.5. Discussion

The main finding of this study is that the combination of plant sterols and glucomannan had a greater cholesterol-lowering effect than plant sterols and glucomannan alone in both type 2 diabetic and non-diabetic mildly hypercholesterolemic individuals. In the present study, 1.8 g/day of plant sterols reduced LDL cholesterol by 8.5 % compared to baseline LDL cholesterol levels by day 7. Although a reduction in LDL cholesterol concentrations was not significant after the first week of plant sterol treatment, the reduction in the first week is comparable to the findings from a recent meta-analysis review (Law, 2002). This study showed 2.0 g/day plant sterols or stanols in margarine decreased LDL cholesterol by 9-14% after 3 weeks. The compliance was assessed by weighing the amount of bars which has its limitations. The plateau effect of LDL cholesterol concentrations in week 2 and 3 may be due to the decreased compliance of the treatment. On the other hand, 10 g/day of glucomannan also reduced plasma LDL cholesterol by 14.5 % in the type 2 diabetic and non-diabetic groups. Results from a similar supplementation study in healthy males, showed that at approximately half the dose amount of glucomannan (3.9 g/day), a 7.2% reduction in LDL cholesterol concentrations was achieved (Arvill and Bodin, 1995). Therefore, it can be speculated that the hypocholesterolemic effect of glucomannan is dose-dependent. In the current study, the combination of plant sterols and glucomannan reduced LDL cholesterol concentrations by 21.4% from baseline (day 0) which is not comparable to the cholesterol-lowering effect of either plant sterols or glucomannan supplementation alone. This demonstrates that plant sterols and glucomannan may lower circulating cholesterol concentrations in an additive manner.

The possible mechanism of this additive cholesterol-lowering effect by combination of plant sterols and glucomannan may involve a reduction in both cholesterol absorption and synthesis. Plant sterols suppress intestinal cholesterol absorption by reducing dietary and biliary cholesterol incorporation into micelles (Ikeda *et al.* 1988a, Ikeda *et al.* 1988b, Ikeda *et al.* 1989, Plat and Mensink, 1999, Nissinen *et al.* 2002). However, it has been shown that the inhibition of the intestinal absorption is partially compensated for by an increase in hepatic cholesterol synthesis (Gylling *et al.* 1999a, Jones *et al.* 2000, Miettinen *et al.* 2000). On the other hand, glucomannan suppresses the postprandial insulin peak by delaying the absorption of nutrients in the small intestine (Doi, 1995, McCarty, 2002) as well as enhancing fecal excretion of neutral sterol and bile acids (Vuksan *et al.* 2000). The reduced postprandial insulin concentrations decrease cholesterol biosynthesis (Jones *et al.* 1993) possibly by reducing HMG-CoA activity (Rodwell *et al.* 1976), which may suppress an increase in cholesterol synthesis induced by plant sterol supplementation. In the current study, the plant sterol mixture contained campesterol. In a steady state plasma campesterol levels can be used as an indicator of cholesterol absorption, but because plasma plant sterol levels are influenced by supplementation they were unable to be used to compare cholesterol absorption efficiency among treatments. However, changes in levels of plasma lathosterol, a cholesterol precursor and an index of cholesterol synthesis (Miettinen *et al.* 1990) support this proposed cholesterol-lowering action by plant sterols and glucomannan. Plasma lathosterol levels (14.24 ± 7.56 %) were increased from baseline with plant sterol treatment, but were decreased after supplementation of the combination treatment of plant sterols (-12.91 ± 9.22 %) and glucomannan (-6.32 ± 7.66 %). These changes in plasma lathosterol levels suggest that glucomannan suppresses the increase of cholesterol

synthesis by plant sterol intake. However, since postprandial insulin concentrations were not determined in the current study, it is unclear if insulin contributed to the reduced cholesterol synthesis during the plant sterol and glucomannan supplementation period.

Overall plasma campesterol levels were lower ($p<0.05$) in individuals with type 2 diabetes (5.77 ± 0.42 μmol/L) compared to non-diabetic individuals (7.59 ± 0.38 μmol/L), which may indicate lower sterol absorption efficiency in type 2 diabetic compared with non-diabetic subjects. This finding is consistent to the results of previous studies (Briones *et al.* 1986, Gylling and Miettinen, 1997). However, plasma lathosterol levels, indicators of cholesterol synthesis, were not different between individuals with type 2 diabetes and non-diabetes, despite higher hepatic cholesterol synthesis reported in type 2 diabetics compared to non-diabetics (Naoumova *et al.* 1996). The mechanisms involved in altered cholesterol homeostasis have not been fully defined. One of the proposed mechanisms targets lowered insulin sensitivity as one of the factors which affects cholesterol homeostasis in diabetes. Despite numerous *in vitro* studies, the role of insulin on cholesterol absorption is still unclear. Some studies report that insulin can suppress cholesterol absorption and consequently increase cholesterol synthesis in the liver (Thompson, 1980, Thompson and Rajotte, 1984), whereas others showed that insulin decreases cholesterol biosynthesis by stimulating LDL receptor activity (Wade *et al.* 1988, Wade *et al.* 1989). In humans, the suppressive cholesterol synthesis activity by insulin in both type 2 diabetic and non-diabetic individuals has been observed (Naoumova *et al.*1996). However, since the reduction of cholesterol synthesis was less in subjects with diabetes, this supports the idea that impaired insulin sensitivity may affect the disturbance in cholesterol homeostasis in individuals with diabetes.

Dietary supplementation of glucomannan did not improve the glycemic control indices of fasting insulin and fructosamine levels. However, the finding is in contrast to previous research showing that glucomannan supplementation improved glycemic control in type 2 diabetics (Huang *et al.* 1990, Doi, 1995, Vuksan *et al.* 2000, Chen *et al.* 2003) and non-diabetics (Scalfi *et al.* 1987). The discrepancies between studies may be explained by differences in study design. While previous studies (Scalfi *et al.* 1987, Huang *et al.* 1990, Doi, 1995, Vuksan *et al.* 2000, Chen *et al.* 2003) supplemented glucomannan in combination with a controlled diet, in the present study, participants were asked to maintain their usual diet in order to determine the effect of glucomannan in the context of a North American lifestyle. Therefore, it is possible that the discrepancy in findings between the present and previous studies may be due to the absence of dietary control. Not only the supplementation of glucomannan but also the improvement in dietary habits may be needed to improve glycemic control.

One possible side effect of this plant sterol and glucomannan supplementation study was found to be that the intake of glucomannan changed intestinal habits. Particularly, it was reported that glucomannan supplementation induced increased stool frequency, softened stool condition, and increased gas production. Similar intestinal changes have been reported when glucomannan was supplemented at 7.8 to 13 g/day (Doi, 1995, Vuksan *et al.* 2000). However, no changes in intestinal habits were observed with smaller doses (3.6-3.9 g/day) of glucomannan (Doi *et al.* 1979, Arvill and Bodin, 1995). Therefore, reported changes in intestinal habits may only occur with larger doses of glucomannan. Besides changes in intestinal movements, it was reported that the palatability of treatment

granola bars with glucomannan was lower ($p<0.05$) compared to plant sterol granola bars. This may be due to the rheological characteristics of glucomannan. Glucomannan is a viscous soluble fiber and when it absorbs water, it forms a highly viscous gel in the mouth. This viscous gel plays an important role in its hypoglycemic action, but it also creates an unpalatable texture. It has also been suggested that palatability is one of the reasons why viscous fibers have had little impact in clinical studies (Aro *et al.* 1981, Vuorinen-Markkola *et al.* 1992). Considering both the possible intestinal side effects and unpalatable texture produced by glucomannan, dosage of glucomannan may play an important role in the acceptance of subjects to comply with the treatment product.

In summary, results of the present study show that both plant sterols and glucomannan lower circulating cholesterol levels, however, a combination of plant sterols and glucomannan had an additive effect and induced a greater reduction in total and LDL cholesterol levels compared to plant sterols or glucomannan alone. Contrary to what was expected, no changes in glycemic control were observed by supplementation of plant sterols and/or glucomannan treatments. Plasma lathosterol levels were increased with the ingestion of plant sterols, however, by adding glucomannan to plant sterols, these changes were reversed. Thus, the combination of plant sterols and glucomannan may be considered an alternative to pharmacological approaches to lower LDL cholesterol levels in mildly hypercholesterolemic individuals with type 2 diabetes and non-diabetes.

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GENERAL CONCLUSION AND SUMMARY

This study examined the effects of plant sterols and glucomannan alone and in combination on plasma lipid concentrations, plant sterol profiles and glycemic controls in type 2 diabetic and non-diabetic subjects. These data are the first to show that supplementation of a combination of plant sterols and glucomannan exerts a greater cholesterol-lowering effect in both type 2 diabetic and non-diabetic individuals than supplementation of plant sterols or glucomannan alone. A combination of plant sterols and glucomannan decreased circulating cholesterol levels in an additive manner without changing other lifestyle habits of the subjects. Based on the results of lathosterol concentrations which are cholesterol kinetic indicators, the reduction of cholesterol levels by the combination of plant sterols and glucomannan is more likely due to the simultaneous suppression of cholesterol absorption and synthesis. However, due to the lack of improvement of glycemic control, it is unclear whether the reduction of cholesterol synthesis is mediated by insulin secretion. In order to see changes in glycemic controls, not only supplementation of plant sterols and glucomannan but also the improvement of other lifestyle factors may be needed.

Future research should examine whether plant sterols and glucomannan synergistically lower circulating cholesterol by simultaneously suppressing both cholesterol absorption and synthesis. Although the participants tolerated the dietary treatments in this study, a number of subjects experienced changes in gastrointestinal symptoms and bowel habits during the glucomannan treatment periods. It is also important to determine the minimum amount of glucomannan required to obtain significant cholesterol-lowering without

producing intestinal side effects. The critical aspect of type 2 diabetes is loss of insulin sensitivity and consequent alteration of cholesterol metabolism. The current study showed the decreased intestinal cholesterol absorption in type 2 diabetes comparing to non-diabetes. Prospective study should focus on the links between alteration of cholesterol homeostasis and development of CVD.

In addition to hypocholesterolemic and hypoglycemic properties, glucomannan has been known to enhance satiety. In the present study, although body weight remained steady during glucomannan treatment, reduction of body weight was observed in type 2 diabetic individuals after the combination treatment. Our subjects also reported that they experienced early satiety after intake of glucomannan. Besides hypoglycemic control, appropriate weight management is essential to prevent the progression of the diabetic condition and its complications. Moreover, weight management is important for non-diabetic individuals who are overweight, because obesity is one the risk factors for developing various diseases such as CVD. Therefore, it may be interesting to investigate its potential role in the suppression of appetite.

Finally, our data demonstrated the potential synergistic cholesterol-lowering actions by plant sterols and glucomannan. Although essential remedies of hypercholesterolemia are the improvement of life style and proper medication, our data provide evidence that supplementation of plant sterols and glucomannan simultaneously possesses the potential to become a convenient alternative for the management and prevention of hypercholesterolemia in type 2 diabetic and non-diabetic individuals.

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Appendix 1. Consent form

Investigation of Plant Sterols and Glucomannans as Cholesterol-Lowering Agents in Subjects with and without Type 2 Diabetes

Patients Name:

School of Dietetics and Human Nutrition, Macdonald Campus of McGill University
Researchers: Dr. Peter Jones, Makiko Yoshida, Phone: 398-7527 day / 457-8641 / eve.
Contact Physician: Dr. William Parsons, Phone: 694-4869

The purpose of the study, the description of the procedure or treatment, its risks and /or benefits, and possible alternative follows:

1. Purpose of the study

The aim of this study is to examine how plant sterols and glucomannan added to the diet influence body level and rate of production of circulating cholesterol. It is known that plant sterols act as cholesterol reducing agents, whereas glucomannan work at the level of improving blood sugar control as well as cholesterol lowering agents. Accordingly, we would like to compare the effect of plant sterols, glucomannan, or combination of each on lipid protein profiles and blood glucose control in diabetic and non-diabetic individuals.

2. Description of the study

Before starting the study, a fasting blood sample 10 ml (2 teaspoons) and a urine sample will be taken for the laboratory to confirm the absence of heath abnormalities and to measure blood lipid profile and glucose. You will be permitted to take stable dose of metformin, sulfonylureas, thyroid hormone, anti-hypertensive agents, oral and post-menopausal estrogens, as long as these are continued equivalently throughout the duration of the period of the study.

During the clinical trial, you will be asked to consume a bar and a 8oz (1 cup) drink three times a day with your regular diet for four 3 week periods (4 X 21days), which will be separated by a 4 week interval when you will consume your usual diet. The bars will be provided weekly in the Clinical Research laboratory. To the bars will be added at either a level of 1.8 g/day a material resembling cholesterol obtained from the pine trees, 10 g/day of soluble fiber, glucomannan, or a combination of plant sterols and glucomannan. These materials are tasteless and odourless.

At the beginning (Day 1) of each treatment period, you will be examined by a physician to ensure that you are in good health. On day 18 of each treatment period, you will be required to take a slice of toast with a spread which contains 15mg of cholesterol with a stable isotope. On the day 21, you will be asked to drink 25ml (1 2/3 tablespoons) water containing stable isotope. On the day 22 (i.e. the morning after the final day of each treatment period), you will be asked to drink 50g (1/5 cup) of glucose and you will provide a blood sample by a finger prick 0, 0.5, 1, 1.5 and 2 hr after the intake of glucose.

Every morning of days 1, 8, 15, 18, 19, 20, 21, and 22 you will provide a fasting blood sample 20ml (1 1/3 tablespoons) for the assessment of cholesterol levels, synthesis rate, as well as blood sugar control. At the end of the each diet period, you will again be examined by a physician to ensure that you are in good heath. Then you will resume consumption of your normal diet until the next diet period. The plant sterol mixture and/or glucomannan will be contained during three of the four diet periods; the other will act as a control period.

3.Potential risk and/or benefit

There are no known hazards associated with the use of the stable labeled tags in the present procedure. A slight chance exists that you will experience transitory dizziness after drinking the labeled water. There are no risks of the procedure other than that normally associated with blood-taking and ingestion of 7C-cholesterol. After blood taking, you may feel dizzy. The plant sterol mixtures and glucomannan added to the diet at the proposed level have been shown to exert no negative effects on health in previous animal and human experiments. When you take glucomannan during the study periods, you may experience flatulence and soft stools. In case you feel any discomfort or any change of your health condition during the experimental trial, a physician, Dr. Parsons will be available to contact at any time. Dr. Parsons can be reached at 694-4869.

Confidentiality

The substance of the project and the procedure associated with it have been fully explained to me, and all experimental procedures have been identified. I have had the opportunity to ask questions concerning any and all aspects of the project and any procedure involved. I am aware that I may refuse to participate as well as withdraw my consent at any time. I acknowledge that no guarantee or assurance has been given by anyone as to results to be obtained. Confidentially of records concerning my involvement in this project will be maintained in an appropriate manner.

Compensation

I understand that, in compensation for the inconvenience of the study schedule, I will receive $ 500 at completion of the trial and subsequently be provided access to my results concerning the lipoprotein and cholesterol synthesis assessment. If I decided to withdraw before completion, or if the study ends early, I will receive an appropriate pro-rated fraction of this amount.

I, _____, the undersigned, Hereby consent to participate as a subject in the above named research project conducted by McGill University.

I acknowledge receiving a copy of this consent form and all appropriate attachments and agree to be contacted by a member of the Research Ethics Committee.

Signature of Subject

Investigator:

Witness:

Date: _____ Time:_____ a.m/p.m

Appendix 2. Questionnaire

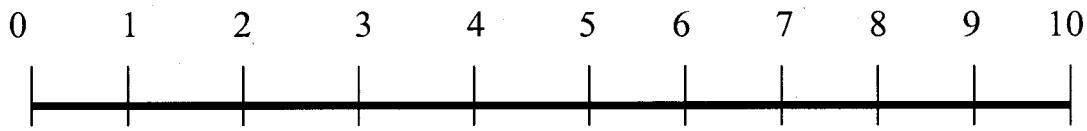
Investigation of Plant Sterols and Glucomannan as Cholesterol-Lowering Agents in Subjects with and without Type 2 Diabetes

School of Dietetics and Human Nutrition, Macdonald Campus of McGill University
Researchers: Dr. Peter Jones, Makiko Yoshida, Phone: 398-7527 day / 457-8641 / eve.
Contact Physician: Dr. William Parsons, 694-4869

Name: Group: Phase:

1. Please circle which number best represent to the taste.

very unpleasant — neutral — very pleasant



2. What do you drink with your bars?

3. Have you noticed any side effects of eating these bars over the 3 wk of period? Yes / No

   If you chose "Yes", Please describe.

4. Did you change your dietary habits during the last 3 week? Yes / No

   If "yes", please describe these changes.

5. Have your bowel movement changed over the last 3 weeks. Yes / No

Stools -------	more often	no change	less often
	harder	no change	softer
	more gas	no change	less gas
	more cramps	no change	less cramps

If changed, in terms of your willingness to regularly consume such nutrient bar, would you say that changes were

insignificant	tolerable	unacceptable

6. Other comments

Appendix 3. Average consumption of granola bars[1]

Consumption (%)	Control	Plant sterols	Glucomannan	Combination
Non-diabetics	98.8 ± 0.006	99.4 ± 0.003	98.5 ± 0.008	99.0 ± 0.008
Type 2 diabetics	97.6 ± 0.008	98.9 ± 0.006	98.4 ± 0.009	98.5 ± 0.013
All	98.3 ± 0.005	99.2 ± 0.003	98.4 ± 0.006	98.8 ± 0.007

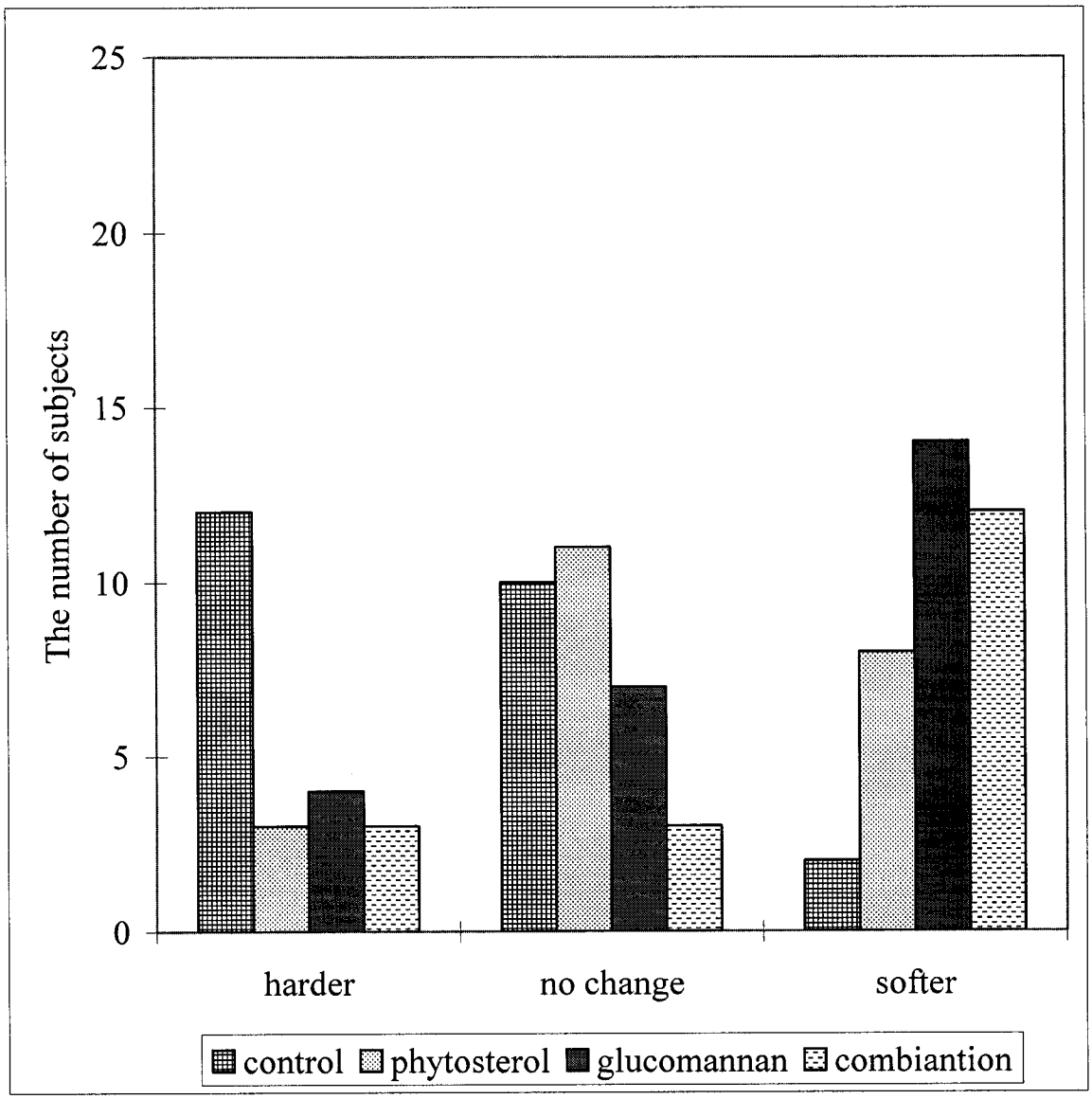
[1]Values are expressed as mean ± S.E.M. Percent change is based on individual data. Percent change relative to control is based on the mean of day 21.

Appendix 4. Palatability of granola bars[1]

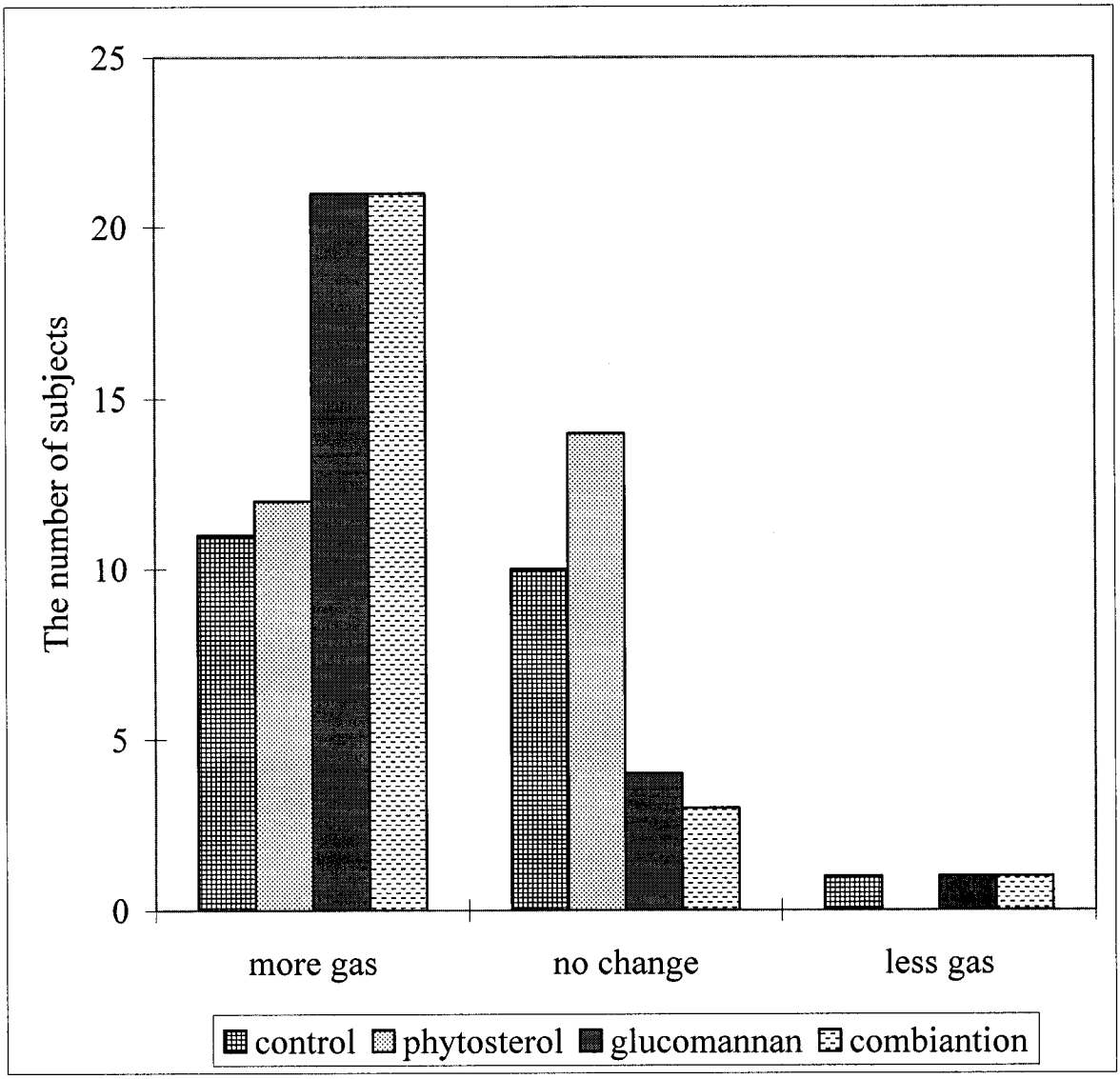
Palatability	Control	Plant sterols	Glucomannan	Combination
Non-diabetics	6.31 ± 0.49	7.00 ± 0.51	5.69 ± 0.61	6.06 ± 0.37
Type 2 diabetics	8.29 ± 0.49**	8.46 ± 0.51	7.25 ± 0.70	6.00 ± 0.71
All	7.16 ± 0.39^{ab}	7.63 ± 0.38^{a}	6.36 ± 0.48^{b}	6.04 ± 0.36^{b}

[1]Values are expressed as mean ± S.E.M. Values carrying different superscript letters indicate a significant difference among treatments ($p<0.05$).
**$P<0.01$; significant difference from non diabetic group.

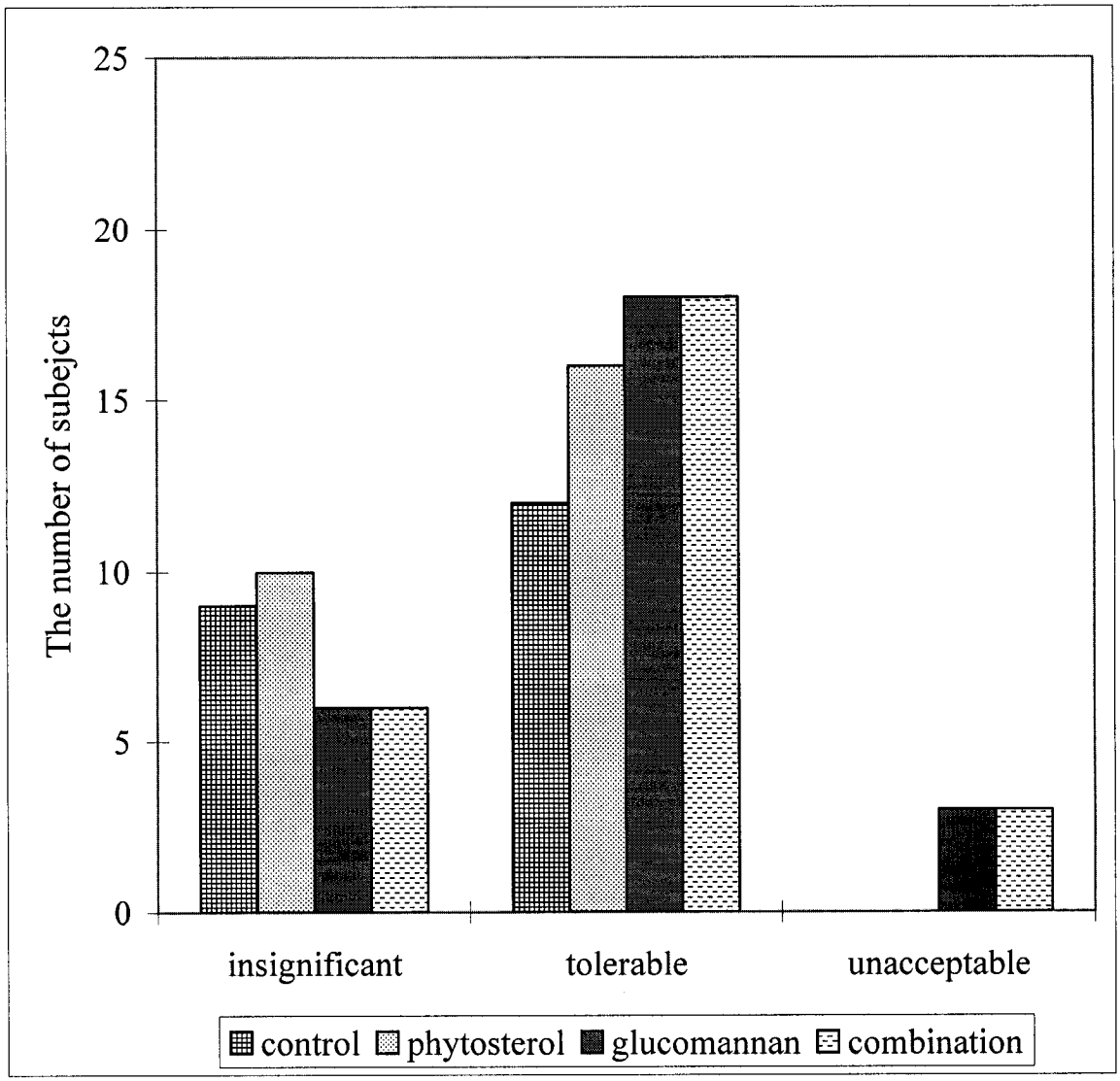
Appendix 5. Stool characteristics in response to treatment



Appendix 6. Gas production in response to treatment



Appendix 7. Intestinal habit changes in response to treatment



Appendix 8. Body weight change at day 0 and day 21 of each treatment period[1]

Body weight (kg)	Control	Plant sterols	Glucomannan	Combination
Non-diabetics				
Day 0	75.28 ± 3.92	75.02 ± 3.64	74.29 ± 3.64	75.02 ± 3.81
Day 21	75.00 ± 3.66	75.15 ± 3.66	74.34 ± 3.59	74.73 ± 3.82
Difference[2]	-0.28 ± 0.44	0.13 ± 0.20	0.50 ± 0.26	-0.29 ± 0.33
% Change	-0.17 ± 0.50	0.17 ± 0.28	0.13 ± 0.33	-0.41 ± 0.39
Type 2 diabetics				
Day 0	80.81 ± 3.05	81.12 ± 2.96	81.54 ± 2.89	81.76 ± 3.00
Day 21	81.69 ± 2.92	81.64 ± 2.77	82.11 ± 2.81	81.68 ± 2.87
Difference[2]	-0.13 ± 0.46	-0.46 ± 0.30	-0.21 ± 0.24	-1.34 ± 0.24*
% Change	-0.14 ± 0.55	-0.48 ± 0.35	-0.26 ± 0.27	-1.61 ± 0.27*
All				
Day 0	77.53 ± 2.65	77.50 ± 2.50	77.24 ± 2.51	77.76 ± 2.61
Day 21	77.72 ± 2.51	77.79 ± 2.48	77.51 ± 2.49	77.56 ± 2.59
Difference[2]	-0.22 ± 0.32	-0.10 ± 0.18	-0.05 ± 0.18	-0.70 ± 0.24
% Change	-0.16 ± 0.36	-0.08 ± 0.22	-0.02 ± 0.23	-0.87 ± 0.28

[1]Values are expressed as mean ± S.E.M. Percent change is based on individual data. Percent change relative to control is based on the mean of day 21.
*P<0.05; significant difference from non diabetic group.
[2]Difference between day 1 and day 21.

Appendix 9. Plasma insulin levels at day 0 and day 21 of each treatment period[1]

Insulin (pmol/L)	Control	Plant sterols	Glucomannan	Combination
Non-diabetics				
Day 0	153.81 ± 18.74	152.81 ± 19.79	141.35 ± 9.97	160.06 ± 13.50
Day 21	158.45 ± 13.50	149.57 ± 10.93	137.86 ± 12.63	157.16 ± 18.15
% Change	13.42 ± 10.15	10.81 ± 10.49	-3.07 ± 4.96	1.91 ± 8.68
% Relative to C[2]		-0.74 ± 6.72	-78.28 ± 36.08	-1.07 ± 6.60
Type 2 diabetics				
Day 0	282.07 ± 24.98***	270.38 ± 28.79**	280.03 ± 32.20***	259.80 ± 33.58**
Day 21	282.58 ± 28.71***	288.27 ± 34.45***	305.55 ± 35.47***	247.60 ± 31.01*
% Change	2.70 ± 6.82	15.09 ± 15.80	19.54 ± 16.45	5.66 ± 14.50
% Relative to C[2]		4.74 ± 11.25	-80.03 ± 35.65	-11.27 ± 6.98
All				
Day 0	211.30 ± 19.21	205.51 ± 19.93	203.52 ± 19.96	204.77 ± 19.93
Day 21	214.10 ± 18.66	211.74 ± 20.82	213.03 ± 23.18	197.70 ± 18.82
% Change	8.62 ± 6.36	12.73 ± 8.98	7.07 ± 7.99	3.59 ± 7.92
% Relative to C[2]		1.71 ± 6.16[b]	-79.7 ± 25.08[a]	-5.64 ± 4.81[b]

[1]Values are expressed as mean ± S.E.M. Values carrying different superscript letters indicate a significant difference between treatments ($p<0.05$). Percent change is based on individual data. Percent change relative to control is based on the mean of day 21. *P<0.05; **P<0.01; ***P<0.001; significant difference from non diabetic group.

[2]Control treatment

Appendix 10. Serum fructosamine levels at day 0 and day 21 of each treatment period[1]

Fructosamine (umol/g)	Control	Plant sterols	Glucomannan	Combination
Non-diabetics				
Day 0	3.38 ± 0.07	3.39 ± 0.07	3.44 ± 0.07	3.48 ± 0.09
Day 21	3.40 ± 0.08	3.38 ± 0.06	3.42 ± 0.06	3.40 ± 0.08
% Change	0.15 ± 0.85	0.08 ± 1.42	-0.33 ± 1.10	-2.02± 1.21
% Relative to [2]C		0.65 ± 1.83	1.26 ± 1.02	1.39 ± 1.58
Type 2 diabetics				
Day 0	4.08 ± 0.18***	4.00 ± 0.19**	4.05 ± 0.16***	4.10 ± 0.17**
Day 21	4.01 ± 0.16**	3.94 ± 0.18**	3.92 ± 0.14***	3.95 ± 0.13**
% Change	-1.42 ± 1.41	-1.32 ± 1.04	-2.87 ± 0.92	-3.28 ± 1.83
% Relative to [2]C		-1.68 ± 1.79	-1.86 ± 0.92	-0.91 ± 2.46
All				
Day 0	3.69 ± 0.11	3.66 ± 0.11	3.71 ± 0.10	3.76 ± 0.11
Day 21	3.68 ± 0.10	3.63 ± 0.10	3.65 ± 0.08	3.65 ± 0.09
% Change	-0.58 ± 0.80	-0.55 ± 0.91	-1.47 ± 0.76	-2.58 ± 1.04
% Relative to [2]C		-0.43 ± 1.28	-0.19 ± 0.74	0.32 ± 1.41

[1]Values are expressed as mean ± S.E.M. Values carrying different superscript letters indicate a significant difference between treatments ($p<0.05$). Percent change is based on individual data. Percent change relative to control is based on the mean of day 21.
**P<0.01; ***P<0.001; significant difference from non diabetic group.
[2]Control treatment

Appendix 11. Blood glucose changes during 2 hour 50g glucose tolerance test