EFFECTS OF PLANT STEROLS AND GLUCOMANNAN ON PARAMETERS OF CHOLESTEROL KINETICS IN HYPERLIPIDEMIC INDIVIDUALS WITH AND WITHOUT TYPE 2 DIABETES

Roula Barake

School of Dietetics and Human Nutrition
McGill University
Montreal, Canada

June 2005

A thesis submitted to the Faculty of Graduate Studies and Research in Partial fulfillment of the requirement of the degree of Master of Science

Your file Votre référence
ISBN: 978-0-494-22703-9
Our file Notre référence
ISBN: 978-0-494-22703-9

For Mom and Dad

ABSTRACT

The objective of this study was to examine the effects of plant sterols and/or glucomannan on lipid profiles and cholesterol kinetics in hyperlipidemic individuals with or without type 2 diabetes. It was hypothesized that plant sterols and glucomannan reduce circulating cholesterol levels and may have an additive or synergistic effect when combined by reducing cholesterol absorption. Thirteen type 2 diabetics and sixteen non-diabetics all mildly hypercholesterolemic free living subjects participated in a randomized crossover trial consisting of 4 phases, 21 days each. Subjects consumed plant sterols and glucomannan during the trial. Overall reductions in total and LDL-cholesterol levels were greater ($P<0.05$) after consumption of the combination supplement. Effects of supplements were not different between diabetics and non-diabetics. No significant changes were observed in cholesterol absorption or synthesis in both diabetics and non-diabetics. The intake of plant sterols and glucomannan together may be an alternative approach in reducing blood cholesterol levels.

RÈSUMÈ

Cette étude a pour objectif d'examiner les effets des stérols de plantes et du glucomannan sur le profile lipidique ainsi que le métabolisme du cholestérol chez les individus hypocholestérolémiques manifestant ou non un diabète de type 2. D'après notre hypothèse, les stérols de plantes et le glucomannan réduisent le taux de cholestérol sanguin et leur combinaison est associée à une synergie de leurs actions respectives sur l'absorption du cholestérol. Treize sujets diabétiques ont participé a cette étude de type croisé, incluant 4 phases de 21 jours chacune. La réduction du taux de cholestérol sanguin observée suite à la consommation du traitement combiné était supérieure ($P<0.05$) à celle observée suite à la consommation des stérols de plantes ou du glucomannan administré individuellement. Aucune différence significative n'a été observée au niveau de l'absorption et de la synthèse du cholestérol à la suite des trois traitements, aussi bien chez les sujets diabétiques que non- diabétiques. Nous concluons qu'une combinaison de stérols de plantes et de glucomannan pourrait être considérée comme une alternative thérapeutique pour la réduction du taux de cholestérol sanguin.

ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisor Dr. Peter Jones, for allowing me to learn from his wealth of knowledge and giving me the opportunity to expand and develop a new area in my quest for knowledge. I would like to thank Dr. Jones for his encouragement and moral support as well as his trust and faith in my abilities that have no doubt boosted my confidence and helped me learn problem solving through different approaches.

I wish to thank my committee members Dr. Kubow for his advice and help. Dr. Kermasha for the lovely chats we had that not only guided and helped me during my studies but were also enlightening for future work.

I would like to extend my gratitude also to all members of the department of Dietetics and Human Nutrition. Especially Lise Grant who made me like the place before I saw it through her emails and made me like it even more through all the help and direction she provided with a smile, no matter how busy she was. Special thanks go to Dr.Gray-Donald, Dr.Starkey, Dr.Thibault and Dr. Koski for their guidance and sincere advice throughout my journey at McGill.

Many thanks go to my lab mates who shared their experience with me; it was a pleasure working with you guys. Special thanks go to my new buddies Romaina and Suhad, who stood by me, shared their knowledge and thoughts. I am honored to have you as friends and hope to stay friends forever. Marie Rached

and AUB girls, you'll always be unique, well, no less is expected from AUB graduates! Helen, Catherine Roynette and Ali thanks for being there for me.

Friends and colleagues in Kuwait and Lebanon, you don't know to what extent your support and care helped me pull through and gave me the extra charge to go on. You will always be in my heart. Aunt Naeemah, I can never thank you enough for your support and presence in my life.

Adib, your support and care meant the world to me, I am glad we were able to reconnect and make up for all the lost time. Reem thanks for your support and love. Bader and Nada the only drawback of getting my Masters is being away from you, you make life worth living.

Last but not least, Mom and Dad; no words can ever express my love, gratitude and appreciation. Every achievement or success in my life is because of all the values you brought me up by, your encouragement, your faith in me and your unconditional love. I dedicate my thesis to you. Thank you very much.

TABLE OF CONTENTS

Abstract..... 3

Résumé..... 4

Acknowledgments..... 5

Table of Contents..... 7

List of Tables..... 9

Contribution of Authors..... 10

Chapter 1. Introduction..... 11

Chapter 2. Literature Review..... 13

2.1. Plant sterol..... 13

2.1.1. Plant sterol as cholesterol lowering agents 13

2.1.2. Plant sterol and mechanism of action..... 14

2.1.3. Plant sterol formulation..... 16

2.1.4. Plant sterol additive effect..... 17

2.1.4.1. Plant sterol with lipid lowering drugs..... 17

2.1.4.2. Plant sterol with viscous fiber..... 19

2.1.5. Plant sterol and diabetes..... 20

2.1.6. Plant sterol safety..... 20

2.2. Glucomannan **24**

2.2.1. Glucomannan and glycemic index 24

2.2.2. Glucomannan and plasma lipids 25

2.2.3. Possible mechanism of action of glucomannan 26

2.2.4. Side effects of glucomannan 27

Rationale **28**

Hypothesis **29**

Objectives **30**

Chapter 3. Manuscript **31**

Effects of plant sterols and glucomannan on parameters of cholesterol kinetics in hyperlipidemic individuals with and without type 2 diabetes

3.1. Abstract 33

3.2. Introduction 35

3.3. Experimental design and methods 36

3.4. Results 42

3.5 Discussion 47

General Summary and Conclusions **57**

Bibliography **60**

Appendices **80**

LIST OF TABLES

Chapter 3. Manuscript

Table1.	Baseline characteristics of subjects.....	51
Table 2.	Plasma lipid concentrations at day 0 and day 21 of each treatment period.....	52
Table 3.	Plasma-non-cholesterol concentrations at day 0 and day 21 of each treatment period.....	54
Table 4.	D7 enrichment as an indicator of absorption and fractional synthesis for all subjects for each dietary period.....	55
Table 5.	D7 enrichment as an indicator of absorption and fractional synthesis for subgroup (A) subjects for each dietary period.....	56

CONTRIBUTION OF AUTHORS

The author of this manuscript was responsible for collection and analyses of cholesterol absorption and synthesis which represent the major component of this manuscript. Cholesterol absorption and synthesis analysis is a multistep procedure that involves extraction, distillation, combustion and use of spectrometry in addition to plasma and standards preparation. The author also was responsible for the statistical analysis and writing the manuscript. Initially, the clinical trial was conducted by a former MSc. student of McGill, Makiko Yoshida, who was investigating the effects of the supplements on lipid profiles and plasma non-cholesterol sterols as well as the effects of the dietary treatments on insulin, fructosamine and oral glucose tolerance test. Her data were submitted in a separate manuscript and accepted in the European Journal of Clinical Nutrition.

Dr. Peter Jones was the principal investigator for the study. He provided the initial structure and supervised all components of the research. Dr. Jones also assisted with the preparation of the manuscript.

CHAPTER 1.

INTRODUCTION

Diseases of the circulatory system account for an appreciable proportion of morbidity and mortality worldwide (Jackson and Lovegrove, 2002). Coronary heart disease (CHD) is a multifactorial condition which has no one single cause; elevated low density lipoprotein (LDL)-cholesterol has been identified as one of the risk factors for developing CHD (Keys, 1970; Willet, 1994). Type 2 diabetes mellitus has been also identified as a risk factor for developing CHD (Kannel and McGee, 1978; Stamler et al, 1993). The risk of developing CHD ranges from 2-7 fold higher in diabetic subjects compared to non-diabetics (Kannel and McGee, 1978; Haffner et al, 1998). Type 2 diabetes is characterized by insulin resistance, impaired β cell function in the pancreas, elevation in total and LDL-cholesterol levels and a reduction in HDL-cholesterol (Selby et al, 1993; Haffner et al, 1998) concentrations.

Scientists have been seeking alternative functional foods based remedies for reducing elevated levels of serum cholesterol and blood glucose in diabetic and non-diabetic individuals (Gylling and Miettinen, 1996; Lee et al, 2003) as a primary preventive measure to reduce CHD.

Plant sterols have been long known for their LDL-cholesterol lowering effect (Katan et al, 2003), safety of consumption of plant sterols has been established (Katan et al, 2003; Ostlund, 2004). Plant sterols have been successfully incorporated with spreads and other functional food forms (Perisee, 2005).

Glucomannan, a soluble fiber obtained from tubers of Amphophallus Konjac, has been accepted as both a hypocholesterolemic and hypoglycemic agent (Vuksan et al, 2000; McCarty, 2002; Chen et al, 2003). The mechanism by which glucomannan is believed to operate is by slowing digestion and absorption rate of glucose (Doi, 1995), which may lead to a reduction in hepatic synthesis of cholesterol via reduction in insulin secretion (Rodwell et al, 1976).

Combining plant sterols and glucomannan in one dietary supplement may exert a more potent effect in reducing LDL-cholesterol as well as improving the glycemic index. Accordingly, aims of the current study are:

1. Assessment of the effects of plant sterols and glucomannan on lipid levels in hypercholesterolemic individuals with type 2 diabetes versus hypercholesterolemic non diabetic individuals.
2. Assessment of whether cholesterol absorption, synthesis and turnover are altered in hypercholesterolemic individuals with type 2 diabetes versus weight matched controls.
3. Determination of whether cholesterol absorption, synthesis and turnover respond to (a) 1.8 g/d of plant sterols, (b) 10 g/d glucomannans, or (c) a combination of the two, differently in hypercholesterolemic individuals with type 2 diabetes versus weight matched control individuals.

CHAPTER 2.

LITERATURE REVIEW

2.1. PLANT STEROLS

Plant sterols are plant-derived compounds that are structurally similar and functionally analogous to cholesterol in humans (Ostlund, 2002). Plant sterols differ from cholesterol by an extra ethyl or methyl group (Lichtenstein, 2002). Humans are incapable of synthesizing plant sterols, and they are poorly absorbed: campesterol is absorbed in a range of 10-15%, sitosterol 4-7%, and absorption of sitostanol, a hydrogenated sitosterol, is about 1%; whereas, 50% of cholesterol is absorbed in the intestinal lumen (Lichtenstein, 2002; Katan et al, 2003; Miettinen et al, 2004; Ostlund, 2004).

2.1.1. PLANT STEROLS AS CHOLESTEROL LOWERING AGENTS

Sterols and stanols appear to be equally effective in reducing LDL-cholesterol whether used in the esterified or unesterfied form and given in doses of about 2.5 g per day (Vanstone et al, 2002; Katan et al, 2003; Ostlund, 2004). It has also been reported that plant stanols from natural sources, namely tall oil, possesses lipid lowering effects even after short term consumption of 10 days; It is worth noting that tall oils had better lowering effect in subjects with higher cholesterol values versus normolipidemic individuals (Jones et al, 1998).

Recent studies showed that smaller doses of plant sterols are effective in reducing blood cholesterol; doses of 0.83, 1.6 or 3.24 grams of plant sterols esters per day had indistinguishable lowering effects on LDL-cholesterol

(Hallikainan et al, 2000). Hallikainan et al (2000), who concluded that 1.6g of plant sterols per day resulted in a maximal LDL-lowering and increasing the dose did not contribute much to further lowering of plasma lipids. In a meta-analysis conducted by Law (2000), it was found that 2 g of plant sterols per day reduced LDL-cholesterol by 0.5 mmol/l for subjects aged from 50-59 years, and 0.4 mmol/l for those who aged 40-49 years. However, the greater reduction in blood cholesterol levels observed in older subjects is due to the fact that older subjects have a higher starting total cholesterol levels.

Total daily plant sterol intake in most studies has been divided into 2 or 3 portions over the day. However, one study reported a similar reduction in LDL-cholesterol levels when 2.5g of plant sterols were given in a single lunch meal or given over 3 meals per day (Plat et al, 2000). The finding of Plat et al (2000) study implies that the distribution of intake over the day may not be an important determinant of efficacy and mechanisms other than replacing cholesterol from mixed micelles for inhibiting cholesterol absorption may be in play (Katan et al, 2003). However, more studies are needed to test the efficacy of plant sterol when given as a single versus more frequent dose.

2.1.2. PLANT STEROLS AND MECHANISM OF ACTION

The primary mechanism by which plant sterols decrease total and LDL-cholesterol is by reducing cholesterol absorption. Several studies have quantified the extent to which sterols (Heinemann et al, 1993; Jones et al, 2000; Vanstone et al, 2002) and stanols (Becker et al, 1993; Miettinen et al, 2000) inhibit intestinal

cholesterol absorption by about 30-50% in vivo using both direct and indirect techniques. This inhibition is partially, but not completely, compensated for by a reciprocal increase in cholesterol biosynthesis rate (Jones et al, 2000; Miettinen et al, 2000; Miettinen, 2001). Naturally occurring plant sterols in corn oil and wheat germ can reduce dietary cholesterol absorption as well (Ostlund et al. 2002; 2003).

Originally, cholesterol absorption was believed to occur by passive diffusion assisted by the concentration gradient of free cholesterol created between the extra and intra cellular space (Wilson et al, 1994; Trautwein et al, 2003). Currently, it is evident that the mechanism involves a much more complex protein-mediated process. The environment of the intestinal lumen consists of an aqueous and oil phase. Cholesterol has to be incorporated into mixed micelles to be absorbed. Micelles are composed fatty acids, free and esterified cholesterol, bile salts and phospholipids. The cholesterol incorporation into the micelles enables it to cross the aqueous barrier of the intestinal lumen. Esterified cholesterol is then hydrolyzed into free cholesterol by pancreatic esterase. Enterocyte membrane proteins, such as scavenger receptor class B type 1(SR-BI) and cell cluster determinant antigen 36 (CD36) may facilitate cholesterol influx into the enterocyte (Werder et al, 2001). ATP binding cassette transporter G5 (ABCG5) and G8 (ABCG8) are transporters, which play a role in sterol homeostasis (Schmitz et al, 2001). The ABCG5/8 proteins pump sterols from the enterocyte back into the intestinal lumen, aiding in regulating sterol absorption (Lee et al, 2001; Schmitz et al, 2001; Gylling et al, 2004). Nonetheless, other

transporters may be more involved in this process and should be further investigated.

Plant sterols are thought to prevent incorporation of free cholesterol into micelles by competing with cholesterol, thus, preventing esterification of free cholesterol into cholesterol ester (Ste-Onge & Jones, 2003; de Jong et al, 2003; Trautwien et al, 2003). Another mechanism by which plant sterols are thought to reduce cholesterol levels is through their effect on ABCG5/8 transporter. It has been reported that consumption of plant sterols may affect the blood cholesterol concentrations in individuals with genetic mutation of this protein (Berge et al, 2000; Weggemans et al, 2002; Plat et al, 2004). However, studies are needed to investigate directly the effect of plant sterols on ABCG5/8 transporter as well as on other proteins involved in cholesterol metabolism such as SR-BI and CD36.

2.1.3. PLANT STEROL FORMULATION

Pure plant sterols have not shown a consistent effectiveness in lowering cholesterol absorption, this finding was attributed to its inert crystalline structure and limited solubility in both water and oil (Ostlund, 2004). Esterifying plant sterols increases their solubility when dissolved in fats such as margarine, salad dressing and butter (Katan et al, 2003). However, foods high in fat content may be of concern for hyperlipidemic subjects since they may add potential risk for developing CHD. As a consequence, scientists have tried to incorporate plant sterols into different low fat food products. In one study, 1g per day of plant stanols added to 0.7% fat yoghurt reduced LDL-cholesterol by 13.7% compared

to control after a 4-week period (Mensink et al, 2002). Interestingly, one study reported that the consumption of 1.8g per day unesterified tall oil derived sterols phytosterol consumed for 4 weeks in a chocolate matrix significantly decreased plasma LDL-cholesterol levels by 10.3%, compared to control (de Graff et al, 2002).

Emulsification of unesterified plant sterols with lecithin in aqueous dispersion has been a second alterative that enables the incorporation of plant sterols in low fat or fat free foods. It has been reported that lecithin-emulsified soy stanols given at 1.9 g per day in non-fat lemonade reduced LDL-cholesterol by 14% (Spillburg et al, 2003). Devaraj et al (2004) has also shown that sterol supplemented orange juice decreased total cholesterol and LDL-cholesterol by 7.2% and 12.4%, respectively, relative to control over a period of 8 weeks. Conversely no changes in changes in LDL-cholesterol were observed when tall oil plant sterols were added to low-fat and fat free beverages (Jones et al, 2003). The difference between Jones et al (2003), Spillburg et al (2003) and Devaraj et al (2004) studies was the presence or absence of lecithin formulation and the degree of micellar phytosterol dispersion. Thus, further work is needed to develop improved manufacturing criteria for better clinical outcomes.

2.1.4. PLANT STEROL ADDITITVE EFFECT

2.1.4.1. PLANT STEROL WITH LIPID LOWERING DRUGS

Blair et al (2000) compared the effect of 5.1g plant stanol ester spreads per day with placebo spreads given to167 subjects on stable doses of statins, drugs that

reduce hepatic cholesterol synthesis, over 8 week period. The authors reported a 17% reduction in LDL-cholesterol compared to a 10% reduction for the placebo group (Blair et al, 2000). Similar findings reported a 20% reduction in LDL-cholesterol when 2.24g plant stanols were added to simvastatin therapy compared to 12% reduction in LDL-cholesterol for those who were not on simvastatin (Vuorio et al, 2000). Gylling et al (1996) also reported 44% reduction in LDL-cholesterol when 3g per day of plant stanol ester margarines were given along with 40 mg/day pravastatin therapy for 7 weeks compared to a 38% reduction in LDL-cholesterol for those who were on pravastatin, but were not consuming plant sterols. Sterols showed similar LDL-lowering effect when added to statin therapy in an 8-week crossover trial (Neil and Miejer, 2001). Musner et al (2002) reported that subjects with higher dietary cholesterol, energy intake, total and saturated fat intake and higher or average baseline cholesterol absorption were better responders to plant sterol esters and statin therapy. Similar findings demonstrated that statin-treated individuals with a high baseline cholesterol absorption rate responded with improved LDL- cholesterol lowering when stanol esters were added to their diets, whereas individuals with low baseline absorption did not (Gylling & Miettinen, 2002, Ostlund, 2004). It is quite evident now that adding plant sterols or stanols appears to be more effective than doubling a statin dose. Doubling statins is known to produce an additional LDL-lowering effect by 5 -7% (Blair et al, 2000) compared to 13% reduction of LDL-cholesterol when statins are coupled with plant sterols or stanols and a 67% reduction in LDL-cholesterol levels when bile acid sequestrants are added together with plant sterols and statins (Gylling & Miettinen, 2002). It is reasonable to speculate that

plant sterols and hypocholesterolemic drugs when combined may have fewer side effects than doubling the drug therapy.

2.1.4.2. PLANT STEROL WITH VISCOUS FIBER

Investigating the efficacy of a combination of soluble fibers along with plant sterols in reducing LDL-cholesterol and whether they can achieve the same magnitude by which statins reduce LDL-cholesterol, Jenkins et al (2003) conducted a clinical trial where 13 subjects consumed a combination of 1.2g/1000 kcal plant sterols, 16.2g/1000 kcal soy protein, 8.3g/1000kcal viscous fiber from barley, oat, and psyllium, 16.6g/1000 kcal almonds with a low saturated fat diet, whereas the control group consumed a low saturated fat diet based on whole-wheat cereal and low-fat dairy foods. A 35% reduction of LDL-cholesterol was reported in the intervention diet to a 12.1% in the control group. The investigators concluded that this combined diet might achieve LDL-cholesterol reductions similar to those achieved by statin therapy (Jenkins et al, 2003). Another double blind, controlled trial involving 112 subjects were randomized to one of 2 treatments; a low fat plus1.8g per day of free tall oil based plant sterol diet and a 2.8g per day of oat β-glucan fiber versus a control diet for duration of six weeks was conducted. No reductions in LDL-cholesterol values for the control group were reported compared to a 3.7% reduction observed in the intervention group (Maki et al, 2003).

2.1.5. PLANT STEROL AND DIABETES

Increased cholesterol synthesis and decreased cholesterol absorption has been reported in type 2 diabetic subjects (Simons et al, 2002) as well as in insulin-resistant normoglycemic subjects (Pihlajamaki et al, 2004). In one study, Gylling et al (1996) reported a marked reduction in LDL-cholesterol (14%) for non-insulin-dependent diabetic subjects consumed plant stanol esters with an inhibition in cholesterol absorption of 68%. A second study involving 2 parallel groups of type 2 diabetic subjects reported a 6.8% reduction in LDL-cholesterol after a 4 week consumption of the intervention diet of 1.6g plant sterols compared to baseline (Lee et al, 2003). It is worth noting that the reduction of LDL-cholesterol became less significant compared to baseline or between the intervention and control group after 8 and 12 weeks of the clinical trial (Lee et al, 2003). Plant sterols may exert a good dietary therapeutic approach for insulin resistant individuals and type 2 diabetic individuals since they reduce cholesterol absorption, however, long term effects may not be maintained. Further studies are needed to confirm these results.

2.1.6. PLANT STEROL SAFETY

The US Food and Drug Administration has accepted that plant sterol/ stanol esters are asserted to be generally recognized as safe (GRAS) by manufacturers. FDA has also authorized a claim that foods containing plant sterol/stanol esters may reduce the risk of coronary heart diseases (CHD) (Federal Register of September 8, 2000 [65 FR 54686]) (Katan et al, 2003). Moreover, the Scientific Committee on Foods of the European Union concluded that plant sterol ester

margarine was safe for human use (http://europa.eu.int/comm/food/fs/sc/out65_en.pdf) (Katan et al, 2003).

Since plant sterols reduce circulating LDL cholesterol levels, and these lipoproteins are considered vehicles that transport newly absorbed vitamins, concerns have been raised about the impact of plant sterols on fat-soluble vitamins. In 2 separate studies no significant effects were observed on circulating levels of vitamin K (Hendriks et al, 1999; Plat et al, 2000). Also, vitamin A (retinol) and vitamin D (calciferol) concentrations were unaffected on average by sterols and stanols (Katan et al, 2003). The effect of 1.5 and 3 g per day of microcrystalline plant sterols on circulating levels of retinol, α-tocopherol and α & β-carotene were investigated; no significant reductions were reported (Christisnsen et al, 2001). Hallikainan et al (2000) also reported no changes in serum retinal, α and β-catorine, and calciferol when plant stanol esters were given in different doses of 0, 0.8, 1.6, 2.4 and 3.2g per day for 4 weeks,however, lycopene levels were significantly decreased in female subjects, whereas lycopene levels did not significantly change in males. Although no significant effect on retinol and α-tocopherol were reported after a consumption of 2g per day plant sterols for 8 weeks, a significant increase in vitamin D levels ($p=0.008$) but not retinol and α-tocopherol, was observed (Vople et al, 2001). In one study where a total of 2.5g of plant stanol esters was consumed once or three times per day, it was reported that the sum of lipophilic hydrocarbon carotenoids was slightly, but not significantly lower, during the one dose treatment, and slightly

and significantly lower after the three dose per day regiment after normalizing the data to LDL-cholesterol levels (Plat et al, 2000). Additional recent studies reported that plant sterol esters reduced the bioavailability of β-carotene and α-tocopherol more than did plant free sterols in normocholesterolemic men (Richelle et al, 2004), whereas Clifton et al (2004) reported that plasma carotenoids reduction was greater with higher intake of plant sterols and reduction was reduced with lower intake of plant sterols, however, the reduction in serum carotenoids was partially reversed by increased fruit and vegetable intake. In another study, it has been shown that baked goods supplemented with plant sterol esters; α-tocopherol and β-carotene were able to reduce plasma LDL-cholesterol while compensating for the potential deficiency of β-carotene regardless of the time of the consumption (Quilez et al, 2003). A third study including 46 hyperlipidemic, free-living subjects who had undergone a randomized, double blinded 3-way clinical trial, consumed sterol free spread, 2.3g sterol ester fortified spread and 2.5g plant stanol fortified spread or a control diet, for 3 weeks. During all three interventions the subjects were advised to eat more than 5 servings of vegetables or fruits per day, of which at least 1 serving was to be carrots, sweet potatoes, pumpkins, tomatoes, apricots, spinach or broccoli. In this investigation, LDL-cholesterol values were reduced and carotenoid plasma concentrations were maintained (Noakes et al, 2002).

Over the short term, the adverse effect of plant sterols on fat soluble vitamins and their precursors remains theoretical and does not have a public health concern

provided that people follow the guidelines regarding consumption of fruits and vegetables, namely five servings of fruits and vegetables one of which from β-carotene rich sources (Yankah et al, 2001).

Despite the previously stated facts, caution is still needed as Sudhop et al (2002) reported that patients with family history of CHD who had undergone a bypass surgery had higher plasma plant sterol levels compared to similar patients with no family history of CHD. Whether this finding is attributed to toxicity of absorbed phytosterols or only a marker indicating that the individuals with family history of CHD have increased cholesterol and plant sterol absorption is yet to be determined (Ostlund, 2004). In one study, 0, 3, 6 and 9 grams of plant sterol esters were given to subjects in an 8 week clinical trial, with no side effects reported (Davidson et al, 2001). Hendriks et al (2003) followed up 185 healthy volunteers who consumed 1.6g of plant sterol esters for 1 year; the authors reported no side effects either. It is worth noting, however, that the stability of stanols and sterols for frying purposes has not been established yet (Katan et al, 2003).

To sum up, consumption of 2g per day of plant sterols has been almost unequivocally established as safe and effective in reducing LDL-cholesterol accompanied, with at least 1 serving of carotene rich source of fruits and vegetables, with almost no side effects.

2.2. GLUCOMANNAN

Amorphophallus Konjac, known as glucomannan, is a tuber of oriental origin that has been used in Japan for over a thousand years and has recently been introduced to the western world. Glucomannan is a polysaccharide consisting of glucose and mannose in a ratio of 3:5 (McCarty, 2002). Glucomannan appears to have a greater potential than other soluble fibers in lowering blood lipids (Garcia et al, 1988; Vuksan et al, 1999; 2000), reducing weight (Vita et al, 1992), improving glycemic index (Wolever et al, 1988) in addition to treating childhood constipation (Loening-Baucke et al, 2004). It has been shown that 1% solutions of glucomannan are about ten-fold more viscous than comparable solutions of guar gum and over a hundred–fold more viscous than pectin solutions which explains why glucomannan may exert more powerful clinical effects (Doi, 1995; Garcia et al, 1988). Although glucomannan is used traditionally in noodle form, it is characterized by being tasteless and odorless which enables its incorporation into different food forms savory or dessert to widen its use and consumption, it is also available commercially in a rubbery jelly form or biscuits as well (Chen et al, 2003).

2.2.1. GLUCOMANNAN AND GLYCEMIC INDEX

Glucomannan has shown a reducing effect on postprandial insulin surge as well as a reduction of about 50% under the insulin curve (Doi, 1995; Hopman et al, 1988). It is believed that the delay in gastric emptying as well as the slowing in digesting and absorbing carbohydrates may account for the reduction in insulin surge. Furthermore, Doi et al (1979) reported a reduction in fasting insulin in type

2 diabetic individuals, when diet was supplemented with glucomannan. These findings were supported with two other recent studies. Glucomannan was given in a dose of 0.7g /100 kcal to 11 hypercholesterolemic, type 2 diabetics in a 2 three week treatment, three weeks for glucomannan and 3 weeks for wheat bran biscuits that served as control diet (Vuksan et al, 1999). The authors concluded that adding glucomannan to diet may ameliorate glycemic control and lipid profile (Vuksan et al, 1999). A second study where 0.5g glucomannan/100 kcal was given to subjects with impaired glucose tolerance in a similar clinical study design, led to similar findings (Vuksan et al, 2000).

2.2.2. GLUCOMANNAN AND PLASMA LIPIDS

A meta-analysis was performed to compare the lipid lowering ability of different soluble dietary fibers form 67 controlled trials (Brown et al, 1999). The author concluded that soluble fiber, given in a dose ranging between 2-10g per day, had a significant but disappointingly small reduction in total and LDL-cholesterol. It is worth noting that this meta-analysis did not include glucomannan as one of the fibers examined. On the other hand, a reduction of about 22% in LDL-cholesterol was reported when glucomannan was the fiber introduced, in a dose of 8-13g per day and 25g per day respectively, to diabetic hyperlipidemic subjects compared to controls (Vuksan et al, 1999; 2000). Studies have been conducted with smaller doses of glucomannan. One study reported a reduction of 20.7% in LDL-cholesterol level compared to control in diabetic hyperlipidemic subjects when 2.4g per day glucomannan was consumed for 28days (Chen et al, 2003). A second study introduced 3.9 g per day of glucomannan in capsular form resulting

in about a 7% reduction in LDL-cholesterol over a period of 4 weeks (Avrill and Bodin, 1995).

2.2.3 POSSIBLE MECHANISMS OF ACTION OF GLUCOMANNAN

The mechanism by which fiber lowers blood lipids remains undefined. However, generalizing from rodent studies fed different soluble fiber such as dietary psyllium and pectin; LDL-cholesterol reduction may be attributed to an increase in 7-α-hydroxylase activity, the rate limiting enzyme for converting cholesterol to bile acids (Horton et al, 1994; Matheson et al, 1995). The reduced insulin surge after consumption of glucomannan, causes an increase in 7-α-hydroxylase activity leading in turn to further depletion of the hepatic cholesterol pool and possible up regulating expression of the hepatic LDL-receptors (Twisk et al, 1995; Wang et al, 1996). Therefore, it is speculated that glucomannan should reduce feedback suppression of this enzyme due to the increased formation of the bile acid.

Moreover, the reduction in postprandial insulin may lead to suppression in hepatic cholesterol synthesis by reducing insulin-induced 3-hydroxy-3-methylglutaryl coenzyme A reductase activity, the rate limiting enzyme in cholesterol synthesis (Rodwell et al, 1976). Another suggested mechanism is the reduction in cholesterol absorption associated with an increased intestinal content viscosity associated with glucomannan consumption (Doi, 1995).

2.2.4. SIDE EFFECTS OF GLUCOMANNAN

Similar to all soluble fiber, glucomannan is believed to increase frequency of bowl movement, stool bulk, soften stool, increase flatulence and cause some gastric discomfort (Chen et al, 2003; Vuksan et al, 1999; 2000). However, it is worth mentioning that these symptoms do not last for more than a few days up to a week. Individuals may tolerate the inconvenience and adapt relatively fast (Chen et al, 2003).

Glucomannan, seem to have very promising therapeutic effects in reducing glycemic index, satiety and weight management, lipid lowering effects and constipation management. The mechanism by which glucomannans are speculated to work need to be further explored as well.

Combining plant sterols that reduce cholesterol absorption and glucomannan that is believed to reduce cholesterol synthesis is an effective way in reducing cholesterol levels.

RATIONALE:

Dislipidemia is a known risk factor for developing CHD. Individuals with type 2 diabetes are known to have altered cholesterol metabolism leading to an increase in circulating total and LDL-cholesterol. Also, hyperlipidemic non diabetic individuals have increased circulating total and LDL-cholesterol. Plant sterols are known to reduce cholesterol absorption in the intestine, but this is compensated for partially by an increase in hepatic cholesterol synthesis. Glucomannans are known to improve glycemic control as well as they play a role in decreasing cholesterol synthesis. Based on this information the potential for synergistic action between the effect of plant sterols decreasing cholesterol absorption and none or semi-digestible carbohydrates such as glucomannans in blunting the surge in cholesterol synthesis is expected.

This study is intended to evaluate effects of plant sterols and glucomannan, alone and in combination, on plasma lipid levels in hyperlipidemic, diabetic and non-diabetic individuals. As well, as it is the intent of the thesis to evaluate the magnitude of this synergy by examining and quantifying the rate of absorption and synthesis of cholesterol in hyperlipidemic diabetic and non-diabetic individuals.

HYPOTHESIS:

The null hypothesis is that glucomannan and or/plant sterols have no effect on parameters of cholesterol kinetics including absorption and synthesis in diabetic type 2 and non-diabetic mildly hypercholesterolemic individuals.

OBJECTIVES

Specific objectives include:

1. Assessment of the effects of plant sterols and glucomannan on lipid levels in hypercholesterolemic individuals with type 2 diabetes versus hypercholesterolemic non diabetic individuals.
2. Assessment of whether cholesterol absorption, synthesis and turnover are altered in hypercholesterolemic individuals with type 2 diabetes versus weight matched controls.
3. Determine whether cholesterol absorption, synthesis and turnover respond to (a) 1.8 g/d of plant sterols, (b) 10 g/d glucomannans, or (c) a combination of the two, differently in hypercholesterolemic individuals with type 2 diabetes versus weight matched control individuals.

CHAPTER 3. MANUSCRIPT

EFFECTS OF PLANT STEROLS AND GLUCOMANNAN ON PARAMETERS OF CHOLESTEROL KINETICS IN HYPERLIPIDEMIC INDIVIDUALS WITH AND WITHOUT TYPE 2 DIABETES

Roula Barake and Peter J.H. Jones

School of Dietetics and Human Nutrition

McGill University,

Ste. Anne-de-Bellevue, Quebec, Canada H9X 3V9

IN PREPARATION FOR SUBMISSION TO

AMERICAN JOURNAL OF CLINICAL NUTRITION

ABBREVIATIONS

ABC: adenosine triphosphate-binding cassette

BMI: body mass index

CHD: coronary heart disease disease

FSR: cholesterol fractional synthesis rate

HbA1c: glycosylated hemoglobin

HDL: high density lipoprotein

IRMS: isotope ratio mass spectrometry

LDL: low-density lipoprotein

3.1. ABSTRACT

Objective: To determine the effects of supplementing the diet with plant sterols and/or glucomannan on lipid profiles and cholesterol absorption and biosynthesis in hyperlipidemic individuals with and without type 2 diabetes.

Design: A randomized crossover clinical trial consisting of 4 phases of 21 days each, separated by 4 week washout period, took place at The Mary Emily Clinical Research Unit of McGill University. Sixteen subjects with type 2 diabetes and 18 non diabetics, all with mild hyperlipidemia aged between 38-74 years, were given supplements of (1.8g/day) plant sterols, (10g/day) glucomannan, a combination of plant sterols and glucomannan or a placebo, each provided in a granola bar form to be consumed 3 times a day. The 4 supplements were randomized over the 4 phases.

Results: Overall plasma cholesterol concentrations were lower ($p<0.05$) after combination treatment compared to control. Overall plasma LDL-cholesterol concentrations were also lower ($p<0.05$) after glucomannan and combination treatments compared to control. Effects of the three treatments on lipid profile were not different between diabetic and non-diabetic groups compared to control. The effects of the different study treatments on average D_7 enrichment in the cholesterol pool ($p=0.074$) or cholesterol fractional synthesis rate ($p=0.269$) were not different from the effect of control. This observation was similar in diabetic and non-diabetic individuals.

Conclusions:

A combination of glucomannan and plant sterols improved plasma LDL-cholesterol concentration, which coincided with a trend in cholesterol absorption reduction due to glucomannan and plant sterol treatment. Incorporation of these dietary constituents in the daily diet may improve lipid profile in diabetic and non-diabetic hyperlipidemic subjects who are at high risk of developing coronary heart disease.

Keywords: plant sterols; glucomannan; mild hypercholesterolemia; type 2 diabetes; lipids; cholesterol absorption; cholesterol synthesis

3.2. INTRODUCTION

Coronary heart disease (CHD) is a leading cause of mortality in western countries and is rapidly increasing in some developing countries as well (Jackson and Lovegrove, 2002; Katan et al, 2003; Devaraj et al, 2004). Several risk factors have been identified for developing CHD (Murray et al, 1997), which is characterized by elevated total and LDL-cholesterol, has been confirmed as an independent risk factor for CHD (Castelli WP, 1984).Type 2 diabetes has been identified as a risk factor for CHD moreover, the incidence of type 2 diabetes has been increasing dramatically in the last few years (Kanaya et al, 2005). Since dislipidemia occurs frequently in type 2 diabetic individuals, leading to increased probability of developing CHD by 3-5 fold, cholesterol lowering should be one of the premier targets in the prevention of CHD.

Investigators have been seeking alternative remedies for reducing cholesterol and elevated blood sugar in diabetic and non-diabetic individuals (Gylling et al, 1996; Lee et al, 2003). Natural compounds such as plant sterols (PS) have been long established as cholesterol lowering agents through their action on reducing cholesterol absorption and have been incorporated with spreads and other functional food forms (Pollack et al, 1981; Pelletier et al, 1995; Jones et al, 1997; Hendriks et al, 1999; Gylling and Miettinen, 1999; Miettinen and Gylling, 2004). Glucomannan, a soluble dietary fiber obtained from tubers of *Amorphophallus Konjac,* has been accepted as both a hypoglycemic and hypocholesterolemic agent (McCarty, 2002; Vuksan et al, 1999; 2000; Gallaher et al 2002; Chen et al, 2003). The mechanism by which glucomannan is believed to operate is by

slowing digestion, which leads in turn to a reduction in the hepatic synthesis of cholesterol via reduction in insulin secretion (Vlahcevic, 1996). Therefore, combining PS and glucomannan is expected to have a synergistic effect on reducing both cholesterol and blood sugar levels in hyperlipidemic diabetic and non-diabetic individuals. Hence, the objective of this study was to examine the effects of PS and glucomannan, alone and in combination on circulating total and LDL-cholesterol, and their effects on cholesterol absorption and biosynthesis in hyperlipidemic individuals with and without type 2 diabetes. Our null hypothesis was that glucomannan and or/plant sterols have no effect on parameters of cholesterol kinetics including absorption and synthesis in diabetic type 2 and non-diabetic mildly hypercholesterolemic individuals.

3.3. EXPERIMENTAL DESIGN AND METHODS

Subjects

A total of 18 non-diabetic (8 men and 10 women) and 16 type 2 diabetic (4 men and 12 women) volunteers with mild hyperlipidemia were recruited through advertisements in local newspapers. These subjects were aged between 38-74 years. All female subjects were post menopausal. Subjects were required to complete a physical examination and provide a medical history prior to their enrollment in the study. Fasting blood and urine samples were collected and screened for biochemical, hematological and urine indices. The inclusion criteria included serum LDL-cholesterol concentrations ranging between 2.7 and 6.0 mmol/L and triglyceride concentrations < 4.0 mmol/L, non-diabetic subjects glucose concentrations < 6.1 mmol/L and > 7.0 mmol/L for the diabetic subjects

with HbA1c concentrations between 6.0 to 9.0%. Subjects receiving insulin therapy or hypocholesterolemic agents were excluded from the study. Subjects with chronic diseases such as renal, pulmonary, biliary, hepatic or gastrointestinal were excluded. Moreover, subjects who underwent coronary artery bypass or other surgical procedures or had a history of myocardial infarction, angina, or congestive heart failure or were chronic users of fiber laxatives of more than 2 doses per week were also excluded. However, subjects were permitted to continue their medication of metformin, sulfonylurea, thyroid hormone, antihypertensive and postmenopausal estrogen drugs during the study.

Experimental design and diets

This study was a randomized crossover clinical trial, where subjects consumed one of 4 different treatments. Each treatment was provided over 21 days. Each phase was separated by a 4 week washout period. Treatments were offered in a granola bar snack form (Forbes Medi-tech Inc. Vancouver, BC, Canada) which subjects consumed 3 times/day along with a 250 ml of a beverage of choice. During each treatment period, subjects consumed their habitual diet in a free-living setup. The subjects were randomized to 1.8 g/day of plant sterols, 10 g/day of glucomannan, a combination of both or no treatment that served as control.

Protocol

Ninety-six hours before the end of each of the 4 phases (i.e., 07:00 hr on day 18), subjects were given an oral dose of 75 mg (Martin et al, 1986; Klag et al 1993) D_7-cholesterol for cholesterol absorption determination. The D_7-cholesterol was

dissolved in 3 ml of warmed margarine, and consumed on a slice of toast. Blood samples were taken at baseline on day 18, as well as fasting samples on days 19, 20, 21, and 22 to monitor enrichment/decay levels (Bosner et al, 1993; Jones et al, 1993). Subjects consumed their test diets as usual over these days. On day 21 of each feeding period, approximately 25 ml of deuterium oxide was given orally to each subject before breakfast (Jones et al, 1996; Jones et al, 1998) and maintenance doses provided thereafter for 24 hours. The change in D_7 enrichment within red blood cell (RBC) free cholesterol was determined as an index of synthesis over days 21 and 22 corresponding to 72 and 96 hours after initial isotope administration.

During each phase, fasting blood samples were taken at days 0 and 21 for plasma lipid profile and sterols analyses. Red blood cells and plasma were separated by a 15 min centrifuging at 15 rpm within 30 min of phlebotomy and then were stored at -20°C until analysis.

Prior to participating in the clinical trial, all subjects gave informed consent; they were also given the opportunity to discuss with the primary investigator, the physician and the study coordinator any queries in mind. Ethical approval for the study was obtained from the Institutional Review Board of the Faculty of Medicine at McGill University.

Analyses

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) (Siedel et al, 1983; Sugiuchi et al, 1955). Plasma LDL-cholesterol concentrations were calculated using the Friedewald equation (Friedewald et al, 1972).

Cholesterol absorption measurement

Lipid was extracted from RBC's in duplicate by using a modified Folch extraction procedure (Folch et al 1957). To separate free cholesterol from cholesteryl ester, thin- layer chromatography was used (20x20 cm, 250 μ; Scientific Adsorbents Inc, Atlanta). The free cholesterol bands were scraped from the silica gel plates and saponified with 0.5 M methanolic KOH to eliminate any possibility of fatty acid contaminates. The free cholesterol extracts were then dried under nitrogen and transferred into 18-cm long combustion tubes (Vycor; Corning Glass Works, Corning, NY) containing 0.6 grams cupric oxide. Pieces of 2-cm silver wires were added to the tubes, and then sealed under vacuum at < 20 mTorr. The single isotope tracer labeled cholesterol samples were then combusted to deuterium enriched water over 4 hours at 520°C. The water was vacuum-distilled into sealed tubes containing 0.06g Zn (Biogeochemical Laboratories, Indiana University, Bloomington, IN) for deuterium enrichment analysis. These tubes were reduced to deuterium-labeled hydrogen gas at 520°C for about 30 min. The deuterium enrichments of free cholesterol were measured by differential isotope

ratio mass spectrometry (IRMS) with the use of a manually operated dual-inlet system with electrical H^{3+} compensation (VG Isomass 903D). Enrichments were expressed relative to standard mean ocean water (SMOW) and a series of standards of known enrichments from the NBS, which were analyzed concurrently on each day of measurement to correct for any variation in linearity of gain of response of the IRMS (Vanstone et al, 2002). The average of 24, 48 and 72 hours D_7 enrichments values obtained were used as an indicator of absorption.

Cholesterol biosynthesis measurement

Cholesterol biosynthesis was determined as the rate of incorporation of deuterium from body water into RBC membrane free cholesterol over a period of 24 hours between 72 and 96 hours at the end of each of the 4 phases. Deuterated water equilibrates quickly between intracellular and extracellular water pools and allows direct determination of cholesterol formation rates (Jeske et al, 1980). Deuterium enrichment was measured in both RBC free cholesterol and plasma water. To determine cholesterol deuterium enrichment, total RBC lipids were extracted and isolated by using the same procedure described earlier.

To measure the deuterium enrichment of plasma water, additional plasma samples were diluted 9-fold with water to reduce deuterium enrichment to within the normal analytic range. As for the baseline samples were 1-fold diluted. Triplicate samples were vacuum-distilled into Vycor tubes containing 0.06g of Zn,

then reduced to hydrogen gas at 520°C for 30min and analyzed by differential IRMS as described earlier.

The cholesterol fractional synthesis rate (FSR) was taken to represent the RBC free cholesterol deuterium enrichment values relative to the corresponding plasma water sample enrichment after correction for the free cholesterol pool. The FSR represented that fraction of the cholesterol pool that was synthesized in 24 hour and was calculated with the following formula (Jones et al, 1993):

$$FSR\ (pool/d) = (\Delta cholesterol / \Delta\ plasma) \times 0.478$$

where (‰) for deuterium cholesterol is the difference between the enriched free cholesterol and plasma water at 96 and 72hours in parts per thousand relative to a SMOW standard. The factor 0.487 reflects the ratio of labeled H atoms replaced by deuterium (22/46) during in vivo biosynthesis (Jones et al, 1993).

Plasma phytosterol concentration measurement

Plasma phytosterol concentrations were determined in duplicate by gas chromatography from the nonsaponifiable plasma lipid (Rathkowsky et al, 1993, Ntanios and Jones, 1998). Briefly, 1.0-ml plasma samples were saponified with methanolic KOH for 1 hour at 100°C; the non-saponifiable material was then extracted with petroleum ether. Samples with 5-α-cholestane added as an internal standard were injected into a gas-liquid chromatograph equipped with a flame ionization detector (HP 5890 Series II; Hewlett Packard, Palo Alto, CA) and

a 30-m capillary column (SAC-5; Supelco, Bellefonte,PA). Detector and injector temperatures were 310 and 300°C, respectively. The duplicate samples were run isothermally at 285°C. Plant sterol peaks were identified by comparison with authenticated standards (Sigma-Aldrich Canada Ltd., Oakville ON, Canada).

Statistical methods

Repeated measures of analysis of variance (ANOVA) using a general linear model was used to determine the differences across treatments, diabetic and non-diabetic groups and interaction between treatments and groups. A greenhouse-Geisser analysis was used to adjust for normality. P value < 0.05 was considered statistically significant. The baseline characteristics were presented as means ± SD. Other data were presented as means ± SEM. Data were analyzed by using SPSS (Version 11.5). A paired t-test was run to look for further associations between treatments.

3.4. RESULTS

Eighteen non-diabetic subjects with mild hyperlipidemia and 16 subjects with type 2 diabetes enrolled in this study. Two subjects, one diabetic and one non-diabetic, withdrew after having the glucomannan treatment due to experiencing gastric discomforts. Three more subjects, 2 diabetic and 1 non diabetic, had to be excluded from the statistical analysis due to poor compliance with the dietary intervention. Compliance was assessed by weighing the returned uneaten portions of the bars. If consumption of treatment bars was less than 50%, subjects were no longer eligible to complete the trial. Baseline characteristics of

subjects are presented in **Table 1**. Glucose, insulin, HbA1c and fructosamine, were higher ($P < 0.05$) in the type 2 diabetic group as compared to the non-diabetic group. Plasma LDL-cholesterol concentrations were lower ($P< 0.01$). Plasma triglyceride levels were higher ($P< 0.01$) in the diabetic group compared with non-diabetic group. Plasma HDL-cholesterol values were similar in both groups. Both groups were characterized by mild hyperlipidemia. Almost all subjects were overweight or obese with a higher ($P< 0.05$) BMI in the diabetic group. It is reasonable to deduce that the diabetic subjects were well controlled based on their HbA1c values (7.02 ± 0.8%). Participants were neither on insulin therapy nor on any hypolipidemic medication. However, their oral hypoglycemic therapy had been continued: 5 subjects were taking metformin, 6 subjects were on combined therapy of metformin and glyburide and 1 subject was on glyburide and losartan (Yoshida et al, 2005).

Circulating lipid levels in response to treatment

Endpoint plasma lipid concentrations of non-diabetic and type 2 diabetic groups during the different treatment periods are shown in **Table 2.** Overall, supplementation of glucomannan combined with plant sterols reduced ($P < 0.05$) plasma total cholesterol concentrations (4.77 ± 0.20 mmol/l) compared to control (5.38 ± 0.18 mmol/l). The overall percentage change of total cholesterol from baseline was also 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 type 2 diabetic and non-diabetic subjects in response to the treatments.

On day 0 of the glucomannan phase, plasma LDL-cholesterol concentrations 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 concentrations compared to control (3.53 ± 0.16 mmol/l) in non-diabetic and type 2 diabetic subjects. Overall plasma LDL-cholesterol concentrations were also lower ($P < 0.05$) after combination treatment compared with plant sterol treatment (3.43 ± 0.13 mmol/l); however, no difference was observed between the glucomannan and combination treatments.

Plasma triglyceride concentrations were not affected by dietary treatments. However, the plasma triglyceride concentrations were consistently higher ($P < 0.05$) in diabetic group than in non-diabetic group during the entire study period. Plasma HDL-cholesterol concentrations did not differ among the treatment periods. There was also no difference in the changes of HDL-cholesterol concentrations between two subject groups (Yoshida et al, 2005).

Plasma non-sterol concentrations in response to treatment

Plasma non-cholesterol sterols in type 2 diabetic and non-diabetic groups are summarized in **Table 3.** On day 21, overall ratio of β-sitosterol to total cholesterol was higher ($P < 0.05$) following plant sterol treatment 1.09 ± 0.18 mmol/mol) compared to the glucomannan treatment (0.70 ± 0.09 mmol/mol). Plasma β-

sitosterol concentrations were not different between type 2 diabetic and non-diabetic groups after each treatment.

The reductions in plasma lathosterol after 21 days were greater ($P < 0.05$) with the combination treatment (-0.10 ± 0.06 mmol/mol) compared with plant sterol treatment (0.17 ± 0.08 mmol/mol). During the control period, plasma lathosterol concentration changes from baseline (day 1) were greater in individuals with type 2 diabetes compared with non-diabetes group (Yoshida et al, 2005).

Cholesterol absorption in response to treatment

Data for 11 diabetic subjects and 16 non diabetic subjects were available for the statistical analysis. Blood samples for 2 subjects were not sufficient or lost in extraction, reduction or distillation. The average D_7 enrichment in the cholesterol pool (‰) was taken at the end of each treatment phase as an average of the 24, 48 and 72 hours measurements and used as an indicator of absorption **(Table 4)**. Statistically significant differences were observed neither across treatments, nor between diabetic and non-diabetic groups (P=0.651). Moreover, the interaction between treatment and group was not significant (P=0.352). Percent changes in cholesterol absorption were -7.1 %, 20.3 % and -22.1% after plant sterol, glucomannan and combination treatments, respectively, relative to control.

Cholesterol biosynthesis in response to treatment

There was neither statistically significant difference of main effects between treatments, nor within the diabetic and non-diabetic group. Also the interaction

between treatment and group was not significant. Percent changes in cholesterol synthesis were - 2.8%, 5.6% and 51.4% for plant sterol, glucomannan and combination treatments, respectively, relative to control, **Table 4**.

Cholesterol absorption and biosynthesis for subgroup (A) relative to treatment

Looking further into the data, it was noticed that 8 subjects did not follow a physiologically logical expected increase in the enrichment level after consuming the cholesterol tracer isotope. The D_7 cholesterol enrichment curve was flat, which lead to the reasonable assumption that these subjects had not consumed the isotope or that the isotope was not properly administered or prepared. Consequently, data for subgroup (A) was created excluding these 8 subjects from the statistical analysis. The average D_7 enrichment in the cholesterol pool as an indicator of absorption for the remaining 19 subjects is found in **Table 5**. Endpoints in cholesterol absorption were not significantly different across treatments (P=0.074), diabetic and non-diabetic groups (P=0.106). Moreover, the interaction between treatments and groups was not significant. Differences in absorption were -19.3%, -20.6% and -32.5%, respectively, for plant sterol, glucomannan and combination treatments relative to control. As for FSR **(Table 5)**, there were no significant differences across treatments, across groups, and the interaction between treatment and group was not significant. Differences in FSR rates relative to controls were 7.6%, 13.6%, and 62.1% for plant sterol, glucomannan and combination treatments, respectively, relative to control.

A paired t-test was carried out to look for further associations between treatments. A significant reduction in cholesterol absorption was found between plant sterol (P=0.040) and combination treatment (P=0.007) versus control. However, no significant reduction in cholesterol absorption was observed for the glucomannan treatment as compared to control. For FSR no significant differences were observed between any of the treatments versus to control.

3.5. DISCUSSION

This is the first study to test the independent and combined actions of plant sterols and glucomannan on circulating lipid levels and cholesterol kinetics. The main finding is the demonstration of the combined effect of plant sterols and glucomannan in lowering circulating plasma total and LDL-cholesterol. It has been reported that plant sterols may reduce LDL-cholesterol by 10-15% (Berger et al, 2004;Katan et al, 2003; Richelle et al, 2004, Vanstone et al 2002, Simons, 2002; Lichtenstein, 2002, Ostlund, 2004) whereas in this trial a combination of both plant sterols and glucomannan had an additive effect on reduction of LDL-cholesterol by 18% relative to control. The lowering in blood cholesterol may have been attributable to the observed trend in cholesterol absorption reduction due to glucomannan and plant sterol treatment. Plant sterols are known to lower blood cholesterol through reducing cholesterol absorption (Lichtenstein, 2002; Ostlund, 2004) while it has been suggested that glucomannan may lower blood cholesterol by reducing cholesterol absorption and synthesis (McCarty, 2002; Rodwell et al, 1976). In the present study glucomannan alone did not reduce

cholesterol absorption and synthesis. However, more human studies are needed to investigate the mechanism by which glucomannan lowers blood cholesterol.

The reduction in LDL-cholesterol by glucomannan is in agreement with some previous work showing a hypocholesterolemic effect of glucomannan. A 3.9g/day glucomannan given for 28 days in capsular form reduced total and LDL-cholesterol by 10% and 7.2% respectively (Avrill and Bodin, 1995). In another study, a consumption of 0.7g/100 kcal/day glucomannan given in a cookie form, reduced total and LDL-cholesterol by 11% and 20%, respectively, relative to control (Vuksan et al,1999). In a third study, subjects consumed glucomannan in a dose of 8-13g/day and a reduction of 12% of total cholesterol and 22% of LDL-cholesterol, relative to control, were observed (Vuksan et al, 2000). Similar results have been also reported by Chen et al (2003) and Gallaher et al (2002) with a smaller dose of 2.4g/day of a mixed capsule of glucomannan and chitosan, a seaweed fiber. In the present study, the reduction of circulating plasma lipids due to glucomannan treatment were accompanied with a trend in reduction in cholesterol absorption shown by a decrease in D_7 enrichment. However, the reduction in absorption observed was not statistically significant. One possible explanation may be attributed to problems with compliance. Glucomannan is known to cause gastric discomfort, flatulence and increased soft frequent fecal output (Vuksan et al, 1999). These undesirable side effects may have caused some subjects to drop out of the study or not consume the full dose provided. Moreover, glucomannan treatment did not score high on a palatability questionnaire given on the last day of each phase as was reported earlier

(Yoshida et al, 2005), which may have led to an incomplete consumption of the glucomannan treatment.

It was expected that introducing glucomannan with or without plant will affect cholesterol kintetics differently between diabetic and non-diabetic groups (Melga et al, 1992; Hopman et al, 1988; Doi et al, 1979). However, in the present study the observed glucomannan effects on blood lipid profiles did not differ between diabetic and non-diabetic subjects, which may be explained by the relatively normal HbA1c values of the diabetic group. It is possible that this particular group of diabetic individuals enrolled in this study were fairly well controlled on their medication and in their diabetes management, thus the diabetic group may not have represented the diabetic state adequately.

Compared to the literature, the efficacy of plant sterols, when given alone, as cholesterol lowering agents was not as powerful as expected. A possible explanation could be attributed to the food matrix it was introduced by. Plant sterols are more effective in reducing LDL-cholesterol when introduced via higher fat containing vehicles such as margarine (-13%) (Jones et al, 2000), milk (-8.6%) and yoghurt (-8.6%) (Patch et al, 2005) as compared to bread (-5.4%) and cereal (-5.4%) (Clifton et al, 2004; Perisee, 2005) in free living men and women. It has also been reported that plant sterols failed to lower LDL-cholesterol when mixed with low fat beverages (Jones et al, 2003). Conversely, lecithin-emulsified stanols delivered in lemonade given at 1.9 g/day reduced LDL-cholesterol levels by 14% (Spillburg et al, 2003). Devaraj et al (2004) has also showed that sterol

supplemented orange juice decreased total cholesterol and LDL-cholesterol by 7.2% and 12.4%, respectively, relative to control. The difference between Jones et al (2003), Spillburg (2003) and Devaraj (2004) studies was the presence or absence of lecithin formulation and the degree of the micellar plant sterol dispersion. Possibly, the food matrix used in this study did not provide the proper solublization that will allow plant sterols to show their optimum efficacy as cholesterol lowering agents.

In summary, a combination of plant sterols and glucomannan demonstrated an additional impact on total and LDL-cholesterol explained through a trend in reducing intestinal cholesterol absorption, compared to the individual actions of plant sterols or glucomannan alone. Contrary to what was expected, glucomannan did not reduce cholesterol synthesis which suggests that glucomannan works through other mechanism. Incorporating a combination of glucomannan and plant sterol into the habitual diet as functional foods may be a safe and effective adjunct or alternative to pharmaceutical therapy for lipid management in subjects with diabetes or with a risk factor for CHD.

Acknowledgements

The authors would like to thank Dr.Romaina Iqbal for her assistance in statistical analysis, and Suhad AbuMweis for her assistance in statistical analysis and guidance in manuscript preparation. This work was supported by Forbes Medi-tech Inc.

Table 1. Baseline characteristics of subjects[1]

Characteristic	Non-diabetic	Diabetic
	(n=16)	(n=13)
Male/Female	7/9	4/9
Ages (y)	55.19 ± 6.91	56.81 ± 10.77
Body mass index (kg/m2)	27.70 ± 4.48	30.96 ± 3.37*
Lipids (mmol/l)		
Total cholesterol	6.05 ± 0.84	5.53 ± 0.84
LDL cholesterol[2]	4.29 ± 0.74	3.53 ± 0.75**
HDL cholesterol[3]	1.11 ± 0.36	0.92 ± 0.16
Triacylglycerol	1.43 ± 0.53	2.69 ± 1.53**
Glucose (mmol/l)	5.11 ± 0.37	8.56 ± 1.44***
Insulin (pmol/l)	21.03 ± 8.40	35.96 ± 15.91**
HbA1c (%)	5.47 ± 0.20	7.02 ± 0.80***
Fructosamine (umol/l)	3.41 ± 0.30	4.09 ± 0.60***

[1]Values are expressed as mean ± S.D. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ significant difference from non diabetic group. (Yoshida et al, 2005)

[2]Low-density lipoprotein cholesterol

[3]High-density lipoprotein cholesterol

Table 2. Plasma lipid concentrations 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 C2		-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 C2		-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 C2		-3.36 ± 2.05 [a]	-9.50 ± 2.48 [ab]	-13.35 ± 3.14 [b]
LDL cholesterol3				
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 C2		-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 C2		-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 C2		-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 C2		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 C2		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 C2		1.64 ± 6.66	-3.55 ± 7.01	0.19 ± 7.91
HDL cholesterol4				
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 C2		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 C2		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 C2		7.59 ± 5.13	3.33 ± 5.37	1.99 ± 5.61

[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. (Yoshida et al, 2005)
*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. Plasma non-cholesterol sterol concentrations at day 0 and day 21 of each treatment period *1*

	Control	Plant sterols	Glucomannan	Combination
B-sitosterol2				
Non-diabetics				
Day 1	0.73 ± 0.10	0.85 ± 0.14	0.65 ± 0.11	0.75 ± 0.13
Day 21	0.78 ± 0.11	1.22 ± 0.28	0.69 ± 0.11	1.06 ± 0.18
Difference[3]	0.05 ± 0.07	0.27 ± 0.13	0.05 ± 0.04	0.31 ± 0.10
Type 2 diabetics				
Day 1	0.82 ± 0.13	0.82 ± 0.18	0.76 ± 0.12	0.79 ± 0.12
Day 21	0.99 ± 0.18	0.95 ± 0.20	0.82 ± 0.16	0.99 ± 0.17
Difference[3]	0.18 ± 0.15	0.13 ± 0.07	0.06 ± 0.08	0.20 ± 0.08
All				
Day 1	0.77 ± 0.08	0.84 ± 0.11	0.70 ± 0.08	0.77 ± 0.09
Day 21	0.88 ± 0.10 *ab*	1.09 ± 0.18 *a*	0.70 ± 0.09 *b*	1.03 ± 0.12 *ab*
Difference[3]	0.11 ± 0.08	0.20 ± 0.07	0.05 ± 0.04	0.26 ± 0.07
Lathosterol2				
Non-diabetics				
Day 1	0.73 ± 0.07	0.82 ± 0.07	1.08 ± 0.13	0.93 ± 0.09
Day 21	0.88 ± 0.08	0.92 ± 0.13	0.94 ± 0.09	0.86 ± 0.09
Difference[3]	0.17 ± 0.08	0.11 ± 0.11	-0.19 ± 0.10	-0.08 ± 0.07
Type 2 diabetics				
Day 1	0.91 ± 0.11	0.79 ± 0.08	0.87 ± 0.13	1.03 ± 0.13
Day 21	0.85 ± 0.13	1.06 ± 0.11	1.03 ± 0.12	0.94 ± 0.05
Difference[3]	-0.13 ± 0.04*	0.25 ± 0.11	0.04 ± 0.11	-0.12 ± 0.12
All				
Day 1	0.80 ± 0.07	0.80 ± 0.05	0.99 ± 0.09	0.97 ± 0.07
Day 21	0.86 ± 0.07	0.98 ± 0.08	0.98 ± 0.07	0.89 ± 0.05
Difference[3]	0.04 ± 0.06 *ab*	0.17 ± 0.08 *a*	-0.09 ± 0.08 *ab*	-0.10 ± 0.06 *b*

[1] Values are expressed as mean ± S.E.M. Values carrying different superscript letters indicate a significant difference between groups ($p < 0.05$). (Yoshida et al, 2005)

*$p < 0.05$; significant difference from non-diabetic group.

[2] non-cholesterol sterol are expressed as mmol per 1 mol cholesterol.

[3] Difference between day 0 and day 21.

Table 4. D7 enrichment as an indicator for absorption and fractional synthesis for all subjects for each dietary period[1]

	Plant sterol	Glucomannan	Combination	Control
Absorption[2]				
D7 (‰)	131.370 ± 15.370	170.049 ± 28.996	110.143 ± 11.896	141.393 ± 25.925
% Change relative to control	-7.1	20.3	-22.1	
Synthesis[3]				
Rate (pool/d)	0.070 ± 0.005	0.076 ± 0.007	0.109 ± 0.021	0.072 ± 0.008
% Change relative to control	-2.8	5.6	51.4	

[1] Values are expressed as mean ± SEM

[2] n = 26, (non-diabetic 16, diabetic 11)

[3] n =25, (non-diabetic 15, diabetic 10)

Table 5. D7 enrichment as an indicator for absorption and fractional synthesis for subgroup (A) subjects for each dietary period[1]

	Plant sterol	Glucomannan	Combination	Control
Absorption[2]				
D7 (‰)	127.451 ± 13.728	125.920 ± 16.431	106.640 ± 16.150	157.890 ± 11.100
% Change relative to control	-19.3	-20.3	-32.5	
Synthesis[3]				
Rate (pool/d)	0.071 ± 0.006	0.075 ± 0.008	0.107± 0.028	0.066 ± 0.008
% Change relative to control	7.6	13.6	62.1	

[1] Values are expressed as mean ± SEM

[2] n = 19, (non-diabetic 10, diabetic 9)

[3] n =18, (non-diabetic 10, diabetic 8)

GENERAL SUMMARY AND CONCLUSIONS

This study examined the plasma cholesterol lowering ability of plant sterols alone, glucomannan alone and plant sterols and glucomannan combined together on cholesterol kinetics in diabetic and non-diabetic free-living subjects. The novelty of approach of the present study was combining both plant sterols and glucomannan matrixed into granola bars and measuring their effects on cholesterol absorption and synthesis using a single-stable-isotope plasma ratio and deuterium uptake method.

The major finding of the study was the effect of both plant sterols and glucomannan on reducing circulating LDL-cholesterol that accompanied a trend in reducing cholesterol absorption, though the reduction in absorption was not statistically significant. Contrary to our expectations, glucomannan supplementation did not reduce cholesterol synthesis, but did reduce LDL-cholesterol levels. No significant differences in levels of LDL-cholesterol were observed between diabetics and non diabetics across all treatments in this study.

Cholesterol kinetics results were limited by the small number of samples remaining for analysis due to loss of several samples during operational procedures (extraction, distillation or spectrometry). The high subject-to-subject variability in the kinetics of cholesterol metabolism may have contributed to the lack of significance.

Glucomannan was given in a dose of 10g/day, which may have contributed to increases gastric discomfort that increased the number of dropouts from the study. In addition, the food matrix that was used in this study may have been not palatable for some subjects leading to reduced compliance.

In the present study a single-stable-isotope method was used to determine cholesterol absorption. The advantage of using this method lies in it being simpler, less costly and involves less ethical considerations. Moreover, this method has been validated and has proven to be a reliable noninvasive and effective method in determining changes in cholesterol absorption (Wang et al, 2004). Despite the validity of this method, a more advanced approach may be taken into consideration in future studies. Gas-chromatography-combustion combined with isotope ratio mass spectrometry (GC-C-IRMS), which involves a minimal sample manipulation, thereby, less human error (Meir-Augenstein, 1999), may have served as a more direct isotopic analyses approach and may be considered the technology of choice in this case.

In conclusion, the present study confirms previous findings of cholesterol lowering effect of plant sterols and glucomannan alone and demonstrates an additive effect of the combination of these food components. Thus, they may be incorporated into functional foods with minimal side effects and safety concerns. Although life style modifications are highly encouraged as a primary strategy in preventing CHD by improving dietary habits and increasing physical activity; incorporating plant sterols and glucomannan in a functional food bar matrix may

be a potent alternative for drug therapy with minimal side effects and a cholesterol lowering effect without many alterations in the individuals' habitual diets.

Biblioghaphy

Avrill A, and Bodin L. Effect of short-term ingestion of konjac glucomannan on plasma cholesterol in healthy men. Am J Clinic Nutr 1995;61:585-589.

Becker M, Staab D, and von Bergmann K. Treatment of severe familial hypercholesterolemia in childhood with sitosterol and sitostanol. J Pediatr 1993; 122:292-296.

Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, Kwiterovich P, Shan B, Barnes R, and Hobbs HH. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000; 290:1771-1775.

Berger A, Jones PJH, and AbuMweis SS. Plant sterols: factors affecting their efficacy and safety as functional food ingredients. Lipids Health Dis 2004; 1: 5-24.

Blair SN, Capuzzi DM, Gottlieb SO, Nguven T, Morgan JM, and Cater NB. Incremental reduction in serum total cholesterol and LDL-cholesterol with the addition of plant stanol ester-containing spread to statin therapy. Am J Cardiol 2000; 86: 46-52.

Bosner MS, Ostlund RE Jr, Osofisan O Jr, Grosklos J, Fritschle C, and Lange LG. Assessment of percent cholesterol absorption in humans with stable isotopes. J Lipid Res 1993; 34:1047-1053.

Brown L, Rosner B, Willett WW, and Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 1999; 69: 30-42.

Castelli WP. Epidemiology of coronary heart disease-the Framingham- study. Am J Med 1984; 76:4 -12.

Chen HL, Sheu WHH, Tai TST, Liaw YP, and Chen YC. Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects-A randomized double blind trial. J Am Coll Nutr 2003; 22:36-42.

Christiansen LI, Lahteenmaki PL, Mannelin MR, Seppanen-Laakso TE, Hiltunen RV, and Yliruusi JK. Cholesterol lowering effect of spreads enriched with microcrystalline plant sterols in hypercholesterolemic subjects. J Lipid Res 2001; 33: 945-955.

Clifton PM, Noakes M, Sullivan D, Erichsen N, Ross D, Annison G, Fassoulakis A, Cehun M, and Nestel P. Cholesterol lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. Eur J Clin Nutr 2004; 58: 503-509.

Cross-over experiments: design, analysis and application. Edited by: David A.Rathkowsky, Marc A. Evans, and Richard Alldredge.Published New York: Marcel Deker, c1993. pp446.

Davidson MH, Maki KC, Umporowizc DM, Ingram KA, Dicklin MR, Schaefer E, Lane RW, Mcnamara JR, Ribaya-Mercado JD, Perrone G, Robins SJ, and Franke WC. Safety and tolerability of esterified phytosterols administerd in reduced-fat spread and salad dressing. J Am Coll Nutr 2001; 20:307-319.

Devaraj S, Jialal I, and Vega-Lopez S. Plant sterol-fortified Orange juice effectively lowers cholesterol levels in mildly hypercholesterolemic healthy individuals. Arterioscler Thromb Vasc Biol 2004; 24:25-28.

Doi, K. Effects of konjac fiber (glucomannan) on glucose and lipids. Eur J Clin Nutr 1995; 49:S190-S197.

Doi K, Matssra M, Kawara A, and Baba S. Treatment of diabetes with glucomannan (Konjac mannan). Lancet 1979; 8123: 987-988.

Folch J, Lees M, and Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226: 497-509.

Friedewald WT, Levy RI, and Frdrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without the use of preparative centrifuge. Clin Chem 1972; 18:499-502.

Gallaher DA, Gallaher CM, Mahrt GJ, Carr TP, Hollingshead CH, Hesslink R Jr, and Wise J. A glucomannan and chitosan fiber supplement decreases plasma cholesterol and increases cholesterol excretion in overweight normocholesterolemic humans. J Am Coll Nutr 2002; 21:428-433.

Garcia MJ, Chalez M, Fauli C, del Pozo Carrascos A, and Ghirardi PE. Physiochemical comparison of the dietary fibers glucomannan, galactomannan, carboxymethylcellulose, pectin and wheat bran. Curr Ther Res 1988; 43:1010-1013.

de Graff J, Nolting RW, Van Dam M, Belsy EM, Kastelein JJP, Pritchard PH, and Stalenhoef AFH. Consumption of tall oil derived phytosterols in chocolate matrix significantly decreases plasma total and low-density lipoprotein-cholesterol levels. Br J Nutr 2002; 88:479-488.

Gylling H, and Miettinen TA. Effects of inhibiting cholesterol absorption and synthesis on cholesterol and lipoprotein metabolism in hypercholesterolemic non-insulin dependent diabetic men. J Lipid Res 1996; 37:1776-1785.

Gylling H, and Miettinen TA. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism 1999; 48:575-580.

Gylling H, and Miettinen TA. Baseline intestinal absorption and synthesis of cholesterol regulate its response to hypolipidemic treatment in coronary patients. Atherosclerosis 2002; 160:477-481.

Gylling H, Hallikainen M, Pihlajamaki J, Agren J, Laakso M, Rajaratnam RA, Rauramaa R, and Miettinen TA. Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. J Lipid Res 2004; 45:1660-1665.

Haffner SM, Mykkanen L, Stern MP, Paidi M, and Howard BV. Great effect of diabetes on LDL size in women than men. Diabetes Care 1994; 17:1164-1171.

Haffner SM, Lehtos S, Ronnemaa T, Pyorala K, and Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998; 339:229-234.

Hallikainen MA, and Uusitupa MIJ. Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am J Clin Nutr 1999; 69: 403-410.

Hallikainen MA, Sarkkinen ES, and Uusitupa MI. Plant stanol esters affect serum cholesterol concentrations of hypercholesterolemic men and women in a dose-dependent manner. J Nutr. 2000 Apr; 130(4):767-76.

Heinemann T, Axtmann G, and Von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. Euro J Clin Invest 1993; 23:827-831.

Hendriks HFJ, Weststrate JA, van Vliet T, and Meijer GW. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1999; 53:319-327.

Hendiks HFJ, Brink EJ, Meijer GW, Princen HMG, and Ntanios FY. Safety of long term consumption of plant sterol esters-enriched spread. Eur J Clin Nutr 2003; 57: 681-692.

Hopman WP, Houben PG, Speth PA, and Larmers CB. Glucomannan prevents postparandial hypoglycemia in patients with previous gastric surgery. Gut 1988; 29: 930-934.

Horton JD, Cuthbert JA, and Spady DK. Regulation of hepatic 7 alpha-hydroxylase expression by dietary psyllium in hamsters. J Clin Invest 1994; 93:2084-2092.

Jackson KG, and Lovegrove JA. Functional foods, blood lipids and coronary heart disease. Food Science and Technology Bulletin: Functional Foods 2002; 1:1-11.

Jenkins DJA, Cyril WC, Kendall WC, Marchie A, Faulkner D, Vidgen E, Lapsley KG, Trautwein EA, Parker TL, Josse RG, Leiter LA, and Connelly PW. The effect of cobmining plant sterols, soy protein, viscous fibers, and almonds in treating hypercholesterolemia. Metabolism 2003; 52:1478-1483.

Jeske DJ, and Dietschy JM. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using H3 water. J Lipid Res 1980; 21:364-376.

Jones PJH, Leitchtenstein CA, Li ZC, and Conner WE. Human cholesterol synthesis measurement using deuterated water: Theoretical and procedural considerations. Arterioscler Thromb Vasc Biol 1993; 13:247-253.

Jones PJH, Papu AS, Hatcher L Li Z-C, Illingworth DR, and Conner WE. Dietary cholesterol feeding suppresses human cholesterol synthesis measured by deuterium incorporation and urinary mevalonic acid levels. Arterioscler Thromb Vasc Biol 1996; 16:1222-1228.

Jones PJH, MacDougall DE, Ntanios F, and Vanstone CA. Dietary phytosterols as cholesterol-lowering agents in humans. Can J Physiol Pharmacol 1997; 75:217-227.

Jones PJ, Howell T, MacDougall DE, Feng JY, and Parsons W. Short-term administration of tall oil phytosterols improves plasma lipid profiles in subjects with different cholesterol levels. Metabolism 1998; 47:451-756.

Jones PJH, Ausmmam LM, Croll DH, Feng JY, Schaefer EA, and Lichtenstein AH. Validation of deuterium incorporation against sterol balance for measurement of human cholesterol biosynthesis. J Lipid Res 1998; 39:1111-1117.

Jones PJH, Ntanios FY, Raeini-Sarjaz M, and Vanstone CA. Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. Am J Clin Nutr 1999; 69:1144-1150.

Jones PJ, Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Feng JY, and Parsons WE. Modulation of plasma lipid levels and cholesterol kinetics by phytosterol versus phytostanol esters. J Lipid Res 2000; 41:697-705.

Jones PJH, Vanstone CA, Raeini-Sarjaz M, and St-Onge MP. Phytosterol in low and nonfat beverages as part of a controlled diet fail to lower plasma lipid levels. J Lipid Res 2003; 44:1713-1719.

de Jong A, Plat J, and Mensink RP. Metabolic effects of plant sterols and stanols (Review). J Nutr Biochem 2003; 14: 362-369.

Kanaya AM, Herrington D, Vittinghoff E, Lin F, Bittner V, Cauley JA, Hulley S, and Barrett-Connor E. Impaired glucose and vascular outcomes in postmenopausal women with coronary artery disease. Ann Intern Med 2005; 142: 813-820.

Kannel WB, and McGee DL. Diabetes and cardiovascular disease.The Framingham Study. JAMA 1978; 241:2035-2038.

Katan MB, GrungySM, Jones PJH, Law M, Miettinen T, and Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin. Proc 2003;78:965-978.

Keys A. Coronary artery disease in seven countries. Circulation 1970; 41:I1-I211.

Klag JM, Ford DE, Maad LA, HE L, Welton PK, Liang KY, and Lerne D. Serum cholesterol in young men and subsequent cardiovascular disease. N Engl J Med 1993; 328:313-318.

Law M. Plant sterol and stanol margarines and health. Br Med J 2000; 320:861-864.

Lee MH, Lu K, Hazard S, Yu H, Shulenin S, Hidaka H, Kojima H, Allikmets R, Sakuma N, Pegoraro R, Srivastava AK, Salen G, Dean M, and Patel SB. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. Nat Genet 2001; 27:79-83.

Lee YM, Haster B, Scherbaum W, and Hauner H. A phytosterol enriched spread improves the lipid profile of subjects with type 2 diabetes mellitus- A randomized controlled trial under free-living conditions. Eur J Nutr 2003; 42:111-117.

Lichtenstein AH. Plant sterols and blood lipid Levels. Curr Opin Clin Nutr Metab Care 2002; 5:147-152.

Loening-Bauke V, Miele E, and Staiano A. Fiber (glucomannan) is beneficial in the treatment of childhood constiopation. J Pediatr 2004; 113:259-264.

Maki KC, Shinnick F, Seeley MA, Veith PE, Quinn LC, Hallissey J, Temer A, and Davidson MH. Food products containing free tall oil-based phytosterols and oat β-glucan lower serum total and LDL-cholesterol in hypercholesterolemic adults. J Nutr 2003; 133:808-813.

Martin MJ, Browner WS, Hulley SB, Kuller LH, and Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361,662 men. Lancet 1986; 328:933-936.

Matheson HB, Colon IS, and Stoty JA. Cholesterol 7-a-hydroxylase activity is increased by dietary modification with psyllium hydrocolloid, pectin, cholesterol and cholestyramine in rats. J Nutr 1995; 12:454-458.

McCarty MF. Glucomannan minimizes the postprandial insulin surge: a potential adjuvant for hepatothermic therapy. Med Hypotheses 2002; 58:487-490.

Meir-Augnestein W. Use of gas chromatography-combustion-isotope ratio mass spectrometry in nutrition and metabolic research. Curr Opin Clin Nutr Metab Care 1999; 2:1-6.

Melga P, Giusto M, Ciuchi E, Giusti R, and Prando R. Dietary fiber in the diabetic therapy of diabetes mellitus. Experimental data with purified glucomannan. Riv European Sci Med Farmacol 1992; 6:367-373.

Mensink RP, Ebbing S, Lindhout M, Plat J, and van Heugten MM. Effects of plant stanol esters supplied in low fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. Atherosclerosis 2002; 160: 205-213.

Miettinen TA, Vuoristo M, Nissinen M, Jarvinen HJ, and Gylling H. Serum, biliary, and fecal cholesterol and plant sterols in cholectomized patients before and during consumption of stanol ester margarine. Am J Clinic Nutr 2000; 71:1095-1102.

Miettinen TA. Cholesterol absorption inhibition: a strategy for cholesterol lowering therapy. Int J Clin Pract 2001; 55:710-716.

Miettinen TA, and Gylling H. Plant stanol and sterol esters in prevention of cardiovascular diseases. Ann Med 2004; 36:126-134.

Murray CJL, and Lopez AD. Mortality by cause for eight regions of the world: global burden of disease study. Lancet 1997; 349: 1269-1276.

Mussner MJ, Parhofer KG, Von Bergmann K, Schwandt P, Broedl U, and Otto C. Effects of Phytosterol ester enriched margarine on plasma lipoproteins in mild to moderate hypercholesterolemia are related to basal cholesterol and fat intake. Metabolism 2002; 51:189-194.

Neil HA, and Meijer GW. Randomized controlled trial of use by hypercholesterolemic patients of a vegetable oil sterol enriched fat spread. Atherosclerosis 2001; 156:329-337.

Noakes M, Clifton P, Ntanios F, Shrapnel W, Record I, and McInerney J. An increase in dietary carotinoids when consuming plant sterols or stanols is effective in maintaining plasma carotenoid concentrations. Am J Clinic Nutr 2002; 75:79-86.

Ntanios F, and Jones PJH. Effects of variable dietarysitostanol concentrations on plasma lipid profile and phytosterol metabolism in hamsters. Biochim Biophys Acta 1998; 1390: 237-244.

Ostlund RE Jr. Phytosterols in human nutrition. Annu Rev Nutr 2002; 22: 533-549.

Ostlund RE Jr, Racette SB, Okeke A, and Stenson WF. Phytosterols that are naturally present in commercial corn oil significantly reduce cholesterol absorption in humans. Am J Clin Nutr 2002; 75: 1000-1004.

Ostlund RE Jr, Racette SB, and Stenson WF. Inhibition of cholesterol absorption by phytosterol-replete wheat germ compared with phytosterol-depleted wheat germ. Am J Clin Nutr 2003; 77: 1385-1389.

Ostlund RE Jr. Phytosterols and cholesterol metabolism. Curr Opin Lipidol 2004; 15: 37-41.

Patch CS, Tapsell LC, and Williams PG. Plant sterol/ stanol prescription is an effective treatment strategy for managing hypercholesterolemia in outpatient clinical practice. J Am Diet Assoc 2005; 105: 45-52.

Pelletier X, Belbraouet S, Mirabel D, Mordret F, Perrin JL, Pages X, and Debry G. A diet moderately enriched in phytosterols lowers plasma cholesterol concentrations in normocholesterolemic humans. Ann Nutr Metab 1995; 39:291-295.

Perisee D. Food fortification with plant sterol/stanol for hyperlipidemia: Management in free-living populations. J Am Diet Assoc 2005; 105: 52-53.

Pihlajamaki J, Gylling H, Miettinen TA, and Laakso M. Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. J Lipid Res 2004; 45:507-512.

Plat J, van Onselen EN, van Heugten MM, and Mensink RP. Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant sterol esters. Eur J Clin Nutr 2000; 54:671-677.

Plat L, Bragt MCE, and Mensink RP. Common sequence variations in ABCH8 are related to plant sterol metabolism in healthy volunteers. J Lipid Res 2004; 46: 68-75.

Pollack, OJ. & Kritchevsky, D. Beta-sitosterol, in *Monographs on Atherosclerosis.* Karger, Basal, New York.1981:48-157.

Quílez J, Rafecas M, Brufau M, García-Lorda P, Megías I, Bulló M, Ruiz JA, and Salas-Salvadó J. Bakery products enriched with phytosterol esters α-tochopherol and β-carotene decrease plasma LDL-cholesterol and maintain β-carotene concentrations in normolipidemic men and women. J Nutr 2003; 133: 3103-3109.

Richelle M, Enslen M, Hager C, Groux M, Tavazzi I, Godin JP, Berger A, Metairon,S, Quaile S, Piguet-Welsch,C, Sagalowics L, Green H, and Fay LB. Both free and esterifies plant sterols reduce cholesterol absorption and the bioavailability of β-carotene and α-tocopherol in normocholesterolemic humans. Am J Clinic Nutr 2004; 80:171-177.

Rodwell VW, Nordstorm JL, and Mitchelen JL. Regulation of HMG-CoA reductase. Adv Lipid Res 1976; 14:1-76.

Schmitz G, Langmann T, and Heimerl S. Role of ABCG1 and other ABCG family members in lipid metabolism. J Lipid Res 2001; 42:1513-1520.

Selby JV, Austin MA, Newman B, Zhang D, Quesenberry CP, Mayer EJ, and Krauss RM. LDL subclass phenotype and the insulin resistance syndrome in women. Circulation 1993; 85:381-387.

Siedel J, Hagele EO, Ziegenhorn J, and Wahlefeld AW. Reagent for enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin Chem 1983; 29:1075-1080.

Simons LA. Additive effect of plant sterol-ester margarine and cerivastatin in lowering Low-Density lipoprotein cholesterol in primary hyperlipidemia. Am J Cardiol 2002; 90:737-740.

Spilburg CA, Goldberg AC, McGill JB, Stenson WF, Racette SB, Bateman J, McPherson TB, and Ostlund RE Jr. Fat-free food supplemented with soy stanol lecithin powder reduce cholesterol absorption and LDL cholesterol. J Am Diet Assoc 2003; 103: 577-581.

Stamler J, Vaccaro O, Neaton JD, and Wentworth D. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care 1993; 16:343-444.

St-Onge MP, and Jones PJ. Phytosterols and human lipid metabolism: Efficacy, safety, and novel foods. Lipids 2003; 38: 367-375.

Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, and Miyauchi K. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. Clin Chem 1995; 41:717-723.

Sudhop T, Sahin Y, Lindenthal B, Hahn C, Luers C, Berthold HK, von Bergmann K. Comparison of the hepatic clearances of campesterol, sitosterol and cholesterol in healthy subjects suggests that efflux transporters controlling intestinal sterol absorption also regulate biliary secreations. Gut 2002; 51:860-863.

Trautwein EA, Duchateau GS, Lin Y, Mel'nikov SM, Molhuizen HO, and Ntanios FY. Proposed mechanism of cholesterol-lowering action of plant sterols. Eur J Lipid Sci Technol 2003; 105:171-185.

Twisk J, Hoekman MF, Lehmann EM, Miejer P, Mager WH, and Princen HM. Insulin suppresses bile acid synthesis in cultured rat hepatocytes by down-regulation of cholesterol 7 alpha-hydroxylase and sterol 27-hydroxlase gene transcription. Hepatology 1995; 21:501-510.

Vanstone CA, Raeini-Sarjaz M, Parsons WE, and Jones PJH. Unesterfied plant sterols and stanols lower LDL-cholesterol concentrations equivalently in hypercholesterolemic persons. Am J Clinic Nutr 2002; 76: 1272-1278.

Vita PM, Restelli A, Caspani P, and Klinger P. Chronic use of glucomannan in the dietary treatment of severe obesity. Minerva Med 1992; 83:139-139.

Vlahcevic ZR. Regulation of cholesterol 7 alpha-hydroxylase by different effectors. Ital J Gastroenterol 1996; 28:337-339.

Volpe R, Niittynen L, Korpela R, Sirtori C, Bucci A, Fraone N, and Pazzucconi F. Effects of yoghurt enriched with plant sterols on serum lipids in patients with moderate hyper cholesterolemia. Br J Nutr 2001; 86: 233-239.

Vuorio AF, Gylling H, Turtola H, Kontula K, Ketonen P, and Miettinen TA. Stanol ester margarine alone and with simvastatinlowers serum cholesterol in families with familial hypercholesterolemia caused by FH-North Karelina mutation. Arterioscler Thromb Vasc Biol 2000; 20: 500-506.

Vuskan V, Jenkins DJA, Spadafora P, Sievenpiper JL, Owen R, Vidgen E, Brighenti F, Josse R, Leiter LA, and Bruce-Thompson C. Knjac-mannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease type 2 diabetes. A randomized controlled metabolic trial. Diabetes Care 1999; 22:913-919.

Vuskan V, Sievenpiper J, Owen R, Swilley JA, Spadafora P, Jenkins DJA, Vidgen E, Brighenti F, Josse RG, Leither LA, Xu Z, and Novokmet R. Beneficial effects of viscous dietary fiber from konjac-mannan in subjects with the insulin resistance syndrome. Diabetes Care 2000; 23: 9-14.

Wang DP, Stroup D, Marrapodi M, Crestani M, Ghalli G, and Chisng J. Transcriptional regulation of the human cholesterol 7 alpha-hydroxylase gene (CYP7A) in HepG2 cells. J Lipid Res 1996; 37:1831-1841.

Wang Y, Vanstone CA, Parsons WD, and Jones PJH. Validation of a single-isotope-labeled cholesterol tracer approach for measuring human cholesterol absorption. Lipids 2004; 39:1-5.

Weggemans RM, Zock PL, Tai ES, Ordovas JM, Molhuizen HO, and Katan MB. ATP binding cassetteG5 C1950G polymorphism may affect blood cholesterol concentrations in humans. Clin Genet 2002; 62:226-229.

Werder M, Han CH, Werhrli E, Bimmler D, Schulthess G, and Hauser H. Role of scavenger receptors SR-BI and CD36 in selective sterol uptake in the small intestine. Biochemistry 2001; 40: 11643-11650.

Wilson MD, and Rudel LL. Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. J Lipid Res 1994; 35:943-955.

Willet WC. Diet and Health: What should we eat? Science 1994; 264:532-537.

Wolever TMS, Jenkins DJA, Ocana AM, Rao VA, and Collier GR. Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. Am J Clinic Nutr 1988; 48: 1041-1047.

Yankah V, and Jones P. Phytosterols and health implications-efficacy and nutritional aspects. Health and Nutrition 2001; 12:899-903.

Yoshida M, Vanstone CA, Parsons WD, Zawistowski J, and Jones PJH. Effect of plant sterol and glucomannan in lipids in individuals with and without type 2 diabetes. Eur J Clin Nutr 2005 (accepted).

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.

Certification Of Ethical Acceptability For Research Involving Human Subjects

McGill

Faculty of Medicine
3655 Promenade Sir William Osler
Montreal, QC H3G 1Y6

Faculté de médecine
3655, Promenade Sir William Osler
Montréal, QC, H3G 1Y6

Fax/Télécopieur: (514) 398-3595

CERTIFICATION OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMAN SUBJECTS

The Faculty of Medicine Institutional Review Board consisting of:

LAWRENCE HUTCHISON, MD	MICHAL ABRAHAMOWICZ, PHD
PATRICIA DOBKIN, PHD	CATHERINE GARDNER, BSC
NEIL MACDONALD, MD	ROBERT L. MUNRO, BCL
ROBERTA PALMOUR, PHD	LUCILLE PANET-RAYMOND, BA
MARGARET SWAINE, BA	

has examined the research project **A12-M96-01A** entitled **"Investigation of Plant Sterols and Glucamannans as Cholesterol-Lowering Agents in Subjects with or without Type 2 Diabetes"**

as proposed by: Dr. Peter J.H. Jones (Applicant) to _____ (Granting Agency, if any)

and consider the experimental procedures to be acceptable on ethical grounds for research involving human subjects.

December 19, 2001 (Date) — Chair, IRB — Dean of Faculty

Institutional Review Board Assurance Number: M-1458

June 16, 2005

To Whom It May Concern:

The purpose of the present letter is to confirm that the co-author Dr. Peter Jones agrees that the candidate (Roula Barake) includes the manuscript entitled *Effects of plant sterols and glucomannan on parameters of cholesterol kinetics in hyperlipidemic individuals with and without type 2 diabetes* in her thesis.

The candidate's roles in this study included all lab analysis of blood samples involving extraction, distillation, combustion and use of spectrometry in addition to plasma and standards preparation. The author also was responsible for the compiling the data, conducting the statistical analysis and writing the manuscript under the guidance of the co-author and made modifications to it in response to his comments.

Roula Barake

I, the co-author, agree that the candidate, Roula Barake, include the manuscript entitled *Effects of plant sterols and glucomannan on parameters of cholesterol kinetics in hyperlipidemic individuals with and without type 2 diabetes* in her thesis.

6/16/05

Peter Jones