EFFECTS OF PLANT STEROLS ON PLASMA LIPID PROFILES, GLYCEMIC CONTROL OF HYPERCHOLESTEROLEMIC INDIVIDUALS WITH AND WITHOUT TYPE 2 DIABETES

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Dedications to my grandmothers and my parents

ABSTRACT

Plant sterols (PS) are effective in reducing plasma lipid concentrations, however, few studies have examined their cholesterol lowering effects in type 2 diabetics. The objective was to assess whether PS consumption alters blood lipid profile in hypercholesterolemic subjects with and without type 2 diabetes. Fifteen control subjects (age = 55.1±8.5yr and BMI = 26.9±3.0kg/m^2) and fourteen diabetic subjects (age = 54.5±6.7yr and BMI = 30.2±3.0kg/m^2) participated in a double-blinded, randomized, crossover, placebo-controlled feeding trial. The Western diet included either 1.8g/d of PS or cornstarch placebo each provided over 21 d separated by a 28 d washout period. Subjects consumed only foods prepared in Mary Emily Clinical Nutrition Research Unit of McGill University. Total cholesterol (TC) decreased ($p<0.05$) from baseline with PS for control and diabetic subjects by 9.7% and 13.6%, respectively. TC decreased ($p<0.05$) from baseline with placebo for control and diabetic subjects by 10.9% and 11.6%, respectively. Non high density lipoprotein cholesterol (non-HDL-C) decreased ($p<0.05$) from baseline with PS for diabetic subjects by 18.5%. Low density lipoprotein cholesterol (LDL-C) levels were reduced ($p<0.05$) from baseline with PS for control and diabetic subjects by 14.9% and 29.8%, respectively. The reduction of LDL-C due to PS alone is greater with type 2 diabetics. There were no significant changes in HDL-C and TG across diets or treatments. It is thus concluded that PS consumption with diet enhances non-HDL-C and LDL-C reduction compared with diet alone in hypercholesterolemic individuals with and without type 2 diabetes. Demonstration for the first time that PS alone are more efficacious in lowering LDL-C and non-HDL-C in diabetic individuals compared to non-diabetics confirm the beneficial effects of PS to help prevent cardiovascular disease (CVD) for this high risk population.

RÉSUMÉ

Les stérols de plante (SP) réduisent efficacement les concentrations de lipides plasmatiques. Cependant, peu d'études ont examiné les effets hypocholestérolémiques des SP chez les diabétiques de type 2. L'objectif de cette étude visait à évaluer si la consommation des SP pouvait modifier le profil lipidique chez des sujets avec hypercholestérolémie avec et sans diabète de type 2. Quinze sujets de contrôle (âge = 55.1± 8.5 ans et IMC = 26.9 ± 3.0 kg/m^2) et quatorze sujets diabétiques (âge = 54.5 ± 6.7 ans et IMC = 30.2 ± 3.0 kg/m^2) ont participé dans une étude double aveugle, prélevée au hasard, croisée, et contrôlée avec un placebo et un régime contrôlé. Le régime incluait soit 1.8 g/ j de SP ou 1.8 g/ j de fécule de maïs (placebo) et durait 21 jours, séparé par une période d'élimination de 28 j. Les sujets devait seulement consommer le régime préparé par l'unité de recherche en nutrition Mary Emily de l'université McGill au cours des phases SP et placebo. Le régime SP montre une réduction ($p<0.05$) du cholestérol total (CT), comparativement à la concentration de départ de 9.7% et de 13.6% chez les sujets contrôles et les diabétiques, respectivement. Avec le placebo, le CT a diminué ($p<0.05$) par rapport à la concentration de départ de 10.9% et 11.6% chez les sujets contrôles et les diabétiques, respectivement. De plus, le cholestérol provenant des lipoprotéines autres que les lipoprotéines de densité élevée (C-non-HDL) a diminué ($p<0.05$) de 18.5% par rapport à la concentration de départ, grâce au régime SP chez les sujet diabétiques. Le cholestérol des lipoprotéines de faible densité (C-LDL) a aussi diminué ($p<0.05$) avec le régime SP, et montre une diminution par rapport à la concentration de départ de 14.9% et 29.8% chez les sujets contrôles et diabétiques, respectivement. La réduction des C-LDL grâce aux seuls SP était plus importante chez les diabétiques. Les niveaux de cholestérol de lipoprotéines de densité élevée et des triglycérides n'ont pas changé significativement au cours des régimes contrôle et SP. Il est donc conclu que la consommation de SP avec une diète équilibrée, accoît la réduction de C-non HDL et de C-LDL comparé à la simple consommation d'une diète équilibrée chez des sujets avec hypercholestérolémie avec et sans diabète de type 2. Ceci démontre aussi pour la première fois que les SP sont plus efficaces pour réduire les C-LDL et les C-non-HDL chez les diabétiques comparés aux non-diabétiques et confirme les effets bénéfiques des SP pour la prévention de maladies cardiovasculaires (MCV) chez cette population à risque élevé.

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CONTRIBUTION OF AUTHORS

The manuscript included as part of this thesis is entitled "Plant sterol consumption lowers plasma total, non-high density and low density lipoprotein cholesterol in hypercholesterolemic individuals with and without type 2 diabetes" and reports the experimental part of the thesis. Contribution of authors to this manuscript follows.

As the first author, I was responsible for writing and formatting this manuscript, in addition to creating tables and figures. Mélanie Journoud and I were research coordinators of the human feeding trial described in this manuscript. I have been extensively involved with the execution of every aspect of this clinical study, completed over an 8-month period. More specifically, I was responsible for subject recruitment, subject screening, selection and randomization, 3-day cycle menu planning, preparation of daily meals in the metabolic kitchen, and subject supervision at the nutrition research unit during meals and blood draws. Christopher Vanstone coded Plant Sterol powder and cornstarch in containers for blinding purposes. I was also responsible for blood draw scheduling and processing of collected samples. Lucie Gravel performed blood draws on subjects. Melanie Journoud and I coded blood samples tubes. I performed all blood lipid and insulin measurements. I also completed sample preparation and gas chromatography plant sterol analyses on plasma, fatty acid analyses on red blood cells presented in this manuscript. In addition, I was responsible for all statistical analysis of the data included in the present manuscript.

Peter Jones and Melanie Journoud provided editorial assistance with the manuscript. Melanie Journoud, as a co-reseach coordinator, was also involved in subject recruitment, feeding and supervision at the nutrition research unit. She also performed

nutrient analysis of 3-day cycle menu. Peter Jones wrote the grant proposals to obtain funding to conduct this human feeding trial; this was done before I started graduate studies. As my supervisor and principal investigator of the project, Peter Jones provided direction to the study.

1. INTRODUCTION

Type 2 diabetes is often associated with metabolic disturbances including hyperinsulinemia, insulin resistance, frequent dyslipidemia, obesity and impaired beta-cell function (Ginsberg, 1991; Polonsky et al., 1996; Am. Diet. Assoc., 1999). Type 2 diabetes is frequently associated with several lipid aberrations, namely, hypertriglyceridemia, elevated very low-density lipoprotein cholesterol (VLDL-C) and reduced high density lipoprotein cholesterol (HDL-C) (Howard, 1987; De Fronzo et al., 1992; Howard and Howard, 1994; Laakso, 1996). However, elevated low-density lipoprotein cholesterol (LDL-C) is only present in two-thirds of dyslipidemic type 2 diabetes (Harris, 1991). Studies have shown that type 2 diabetes have increased synthesis and decreased absorption of cholesterol (Briones et al., 1986; Gylling and Miettinen, 1994; Gylling et al., 1997; Strandberg et al., 1996; Simonen et al., 2000). As a result, the risk of developing atherosclerotic vascular diseases such as cardiovascular diseases (CVD) is two- to seven- fold higher in type 2 diabetic compared to non-diabetic individuals (Stamler et al., 1993; Strandberg et al., 1996; Haffner et al., 1998).

Diabetic patients without a history of heart disease and non-diabetics with a history of myocardial infarction are equally at risk of suffering an infarction (Haffner et al., 1998). Thus, the Canadian Medical Association published a guideline in which the target blood lipid levels are stricter for diabetic than non-diabetic individuals (Meltzer et al., 1998). Type 2 diabetics are now automatically considered to be in the highest risk category. The target values should, therefore, be as follows: LDL-C of 2.5mmol/L or

less; triglyceride (TG) of 2.0mmol/L or less; and a total cholesterol/HDL-C ratio of 4.0 or less (Hallé, 2000).

Plant sterols, chemically resemble cholesterol, have been shown to block absorption of dietary and endogenously-derived cholesterol from the gut, while being only minimally absorbed themselves (Jones et al., 1997). Because plant sterols are bound to the fibers of the plant, they are difficult to absorb during the transit of digested food through the gut (Bouic, 1998). Daily consumption of moderate quantities of plant sterols, 0.7-3.2g per day, has been shown to consistently reduce plasma total cholesterol by 5-13%, and LDL-C by 6-24% in both normo- and hypercholesterolemic individuals with (Gylling and Miettinen, 1994; Gylling and Miettinen, 1996; Law, 2000; Lee et al., 2003) and without type 2 diabetes (Miettinen et al., 1995; Jones et al., 1997; Jones and Ntanios, 1998; Jones et al., 1999; Hendriks et al., 1999; Pelletier et al., 1995, Weststrate and Meijer, 1998; Hallikainen et al., 2000; Jones et al., 2000; Law, 2000; Plat et al., 2000; Tammi et al., 2000; Nigon et al., 2001, Christiansen et al., 2001).

The efficacy of plant sterols in diabetic subjects compared to non-diabetics has not been well established. Also, the effects of plant sterols in altering the cholesterol absorption and synthesis have not been fully examined in type 2 diabetics. Further investigation is essential in defining dietary strategies for best normalizing risk of CVD and associated complications in type 2 diabetes. Therefore, the objectives of this study were (1) to compare the plasma lipid profiles and glycemic control in hypercholesterolemic individuals with and without type 2 diabetes and (2) to examine

the effects of feeding plant sterols on plasma lipid profiles and glycemic control in hypercholesterolemic individuals with and without type 2 diabetes consuming controlled solid foods diets.

2. LITERATURE REVIEW

2.1. Abnormal Lipid, Altered Cholesterol Metabolism in Type 2 Diabetes

2.1.1. Type 2 Diabetes: an Overview

Type 2 diabetes mellitus, also known as non-insulin dependent diabetes mellitus (NIDDM), is one of the leading public health problems in Canada and in other industrialized countries. Approximately 150 million people around the world are estimated to have diabetes, including approximately 1.25 million Canadians and about 90% of these people have type 2 diabetes (Rowe, 2000). The prevalence of type 2 diabetes in individuals 40 years of age and older who see a general practitioner is 16.4% in Canada and nearly 20% in Quebec, according to Diabetes Screening in Canada (DIASCAN) study (Leiter et al., 1998).

Type 2 diabetes is associated with metabolic disturbances including hyperinsulinemia, insulin resistance, frequent dyslipidemia, obesity and impaired beta-cell function (Ginsberg, 1991; Polonsky et al., 1996; Am. Diet. Assoc., 1999). Other factors such as genetic and environmental factors have also been attributed as predisposing factors to type 2 diabetes (Polonsky et al., 1996). Insulin resistance involves glucose intolerance, abdominal obesity, hypertension, hypertriglyceridemia, and low HDL-C, whereas isolated hypercholesterolemia is not considered an insulin resistant state (Strandberg et al., 1996). All of these possible features of type 2 diabetes increase the risk of atherosclerotic vascular diseases such as CVD.

2.1.2. Role of Insulin in Abnormal Lipid Metabolism in Type 2 Diabetes

Due to insulin resistance, type 2 diabetes is often associated with an absolute increase in the level of plasma insulin, particularly in the postprandial state (Howard, 1994). This hyperinsulinemia leads to abnormalities of lipoprotein metabolism which are responsible for the hyperlipidemia characteristic of the diabetic states (Gibbons, 1989).

Insulin affects a number of processes involved in mammalian lipid metabolism. Insulin stimulates the de novo synthesis of fatty acids in liver, adipose tissue, intestine and lactating mammary gland. The activity of lipoprotein lipase in white adipose tissue is increased by insulin. Thus, it promotes the uptake and re-esterification of non-esterified fatty acids (NEFA) released from plasma TG whereas chylomicrons derived from the intestine and VLDL from the liver. Insulin ensures the storage of NEFA in the adipose tissue depots (Williamson, 1989).

Moreover, insulin plays a key role in the co-ordination of hepatic VLDL lipoprotein metabolism. TG synthesized in the liver from dietary carbohydrate is secreted into plasma and delivered to peripheral tissues as VLDL (Gibbons, 1989). Fatty acids are then derived from the VLDL-TG by the action of lipoprotein lipase in these peripheral tissues, particularly in the muscle and adipose tissue sites (Gibbons, 1989). In muscle, fatty acids may be utilized as a source of energy, whereas in adipose tissue they are re-esterified and stored as TG (Gibbons, 1989).

Type 2 diabetes is frequently associated with several lipid aberrations, namely, hypertriglyceridemia, elevated VLDL-C and reduced HDL-C. However, elevated LDL-C was only present in two-thirds of dyslipidemic type 2 diabetes (Harris, 1991). In clinical practice, normal LDL-C levels are frequently seen in type 2 diabetes, where the response is quite unexpected in light of their high CHD risk. Therefore, recent literature indicated compellingly that non-HDL cholesterol (non-HDL-C) is a comparatively better predictor of CHD risk than LDL-C, especially for type 2 diabetes (Cui et al., 2001). Both hyperglycemia and dyslipidemia are involved in the development of diabetic complications (De Fronzo el al., 1992; Laakso, 1996).

Diabetic patients without a history of heart disease and non-diabetics with a history of myocardial infarction are equally at risk of suffering an infarction (Haffner et al., 1998). Thus, the Canadian Medical Association published a guideline in which the target blood lipid levels are stricter for diabetic than non-diabetic individuals (Meltzer et al., 1998). Type 2 diabetics are now automatically considered to be in the highest risk category. The target values should, therefore, be as follows: LDL-C of 2.5mmol/L or less; TG of 2.0mmol/L or less; and a total cholesterol/HDL-C ratio of 4.0 or less (Hallé, 2000).

2.1.3. Altered Absorption and Synthesis of Cholesterol in Type 2 Diabetics

Type 2 diabetes is characterized by deranged glucose metabolism which is often associated with obesity, especially of the abdominal type and hypertriglyceridemia (Bjorntorp, 1988; Howard and Howard, 1994). Strandberg et al. (1996) showed that type

2 diabetes clearly has increased cholesterol precursors and sterol ratios, reflecting increased cholesterol synthesis. As well, the respective cholestanol and plant sterol ratios, reflecting cholesterol absorption, were decreased in relation to increasing fasting blood glucose (Strandberg et al., 1996). This relation was found to be long lasting. However, increased synthesis and decreased absorption of cholesterol have been reported only in hypertriglyceridemic type 2 diabetics (Briones et al., 1986). There is no clear explanation from literature why individuals with type 2 diabetes have increased synthesis and decreased absorption. As mentioned, type 2 diabetes is often associated with insulin resistance and mild obesity. These two factors may contribute to lower the cholesterol absorption because they change the cholesterol concentration present in the body and change the absorption mechanism at the intestinal level (Simonen et al., 2000). The main factors known to interfere with cholesterol absorption efficiency are: dietary plant sterols, fat and cholesterol, obesity, intact bile acid metabolism, adequate amount of absorptive intestinal epithelium, intestinal transit time, apo E phenotype distribution, and serum TG levels (Bennion and Grundy, 1977; Abrams et al., 1977; Saudek and Brach, 1978; Ponz de Leon et al., 1982; Kesaniema et al., 1987; McNamara et al., 1987; Farkkila at al., 1988; Miettinen and Kesaniemi, 1989; Gylling and Miettinen, 1992; Sutherland et al., 1992). Although obesity modifies digestion processes (Wisen and Johansson, 1992), it is not known to inhibit sterol absorption, and normal range of blood glucose without diabetes in not known to shorten intestinal transit (Strandbery et al., 1996). Other aforementioned factors were unrelated to the glucose level. Moreover, due to the homeostatic regulation of cholesterol metabolism, low intestinal absorption of cholesterol up-regulates cholesterol synthesis and turnover (Gylling and Miettinen,

1997). It is thus believed that enhanced cholesterol synthesis in type 2 diabetics may in part be due to loss of sensitivity to the inhibitory effect of insulin (Naoumova et al., 1996). In other words, insulin resistance could increase hepatic lipid synthesis, VLDL production, biliary cholesterol secretion, and apo B receptor activity (Abrams et al., 1982; Miettinen, 1988; Kesaniemi and Grundy, 1983). It was also found that type 2 diabetics have an increase in hepatic LDL removal coupled with a higher hepatic apo B (LDL) receptor activity, and some increase in lipoproteins containing LDL-C and TG (Gylling and Miettinen, 1997; Simonen et al., 2000). In conclusion, increased cholesterol synthesis and turnover and lowered cholesterol absorption should be added as new features to the metabolic syndrome of type 2 diabetes.

2.2 Effects of Plant Sterols

2.2.1. Plant sterols: an Overview

Plant sterols are naturally occurring constituents of all plants, including fruits and vegetables (anonymous, 2001). Saturation of plant sterols at the 5-alpha position forms compounds including sitostanol and campestanol. Plant sterols, although structurally similar to cholesterol, are not synthesized by the human body. Moreover, plant sterols, chemically resembling cholesterol, inhibit the absorption of cholesterol in the gut. PS added to diet are poorly absorbed by the human intestine (Bouic, 1998).

2.2.2. Effects of Plant Sterols on Blood Lipid Profile

Plant sterols are useful hypocholesterolemic agents. A review of 14 randomized double-blinded trials demonstrated that a daily intake of about 2g of PS lowers the LDL-

C concentrations by 10-15% (Law, 2000). Therefore, differences in efficacy may also be related to factors such as the nature and form of the plant sterols used, the dose, the background diet and subject variability in age and in efficiency of cholesterol absorption (Miettinen and Gylling, 1999).

Sitostanol, the saturated derivative of the most common plant sterol, beta-sitosterol, has successfully been shown to lower circulating cholesterol concentrations in most human feeding trials (Weststrate and Meijer, 1998). Vanstone et al. (2002) indicated that consumption of both sterol- and stanol-containing mixtures are as effective in reducing circulating cholesterol level.

Miettinen et al. (1995) reported that, although consumption of 2.6g/d vs. 1.8g/d of sitostanol ester for 6 months reduced plasma cholesterol levels to a greater extent, both doses were equally effective from a practical point of view. Other studies have shown the dosage-response relationship of plant sterol in cholesterol lowering action. Hendriks et al. (1999) showed that the quantity of daily consumption of sterols and their derivatives is considerably lower than previously thought to have significant cholesterol-lowering effects. In a randomized cross-over trial, different doses of sterol esters, 0.83 to 3.24g/d, were given to normocholesterolemic subjects for 3.5 weeks. The dosage of 1.6 g/d of sterol esters produced significant declines in both total and LDL-C levels compared to the other phases (Hendriks et al., 1999). Hallikainen et al. (2000) showed similar results regarding the dose-response relationship with hypercholesterolemic

individuals. The most recent dosage of more than or equal to 1.5g/d of plant sterols exerted effective cholesterol reduction (Mensink et al., 2002).

Jones et al. (2000) showed that, under controlled dietary conditions, plant sterol esters possess improved efficacy in reducing plasma total and LDL-C concentrations compared with stanol esters at similar intake levels. First, the controlled diet used was relatively high in monounsaturated and polyunsaturated fats, which may have replaced the saturated fats typically found in subjects' habitual intakes. Second, this control diet had a lower cholesterol content as a result of the unsaturated fat. Third, the diet was fed to avoid a positive energy balance, a metabolic state associated with increased circulating insulin concentrations and cholesterogenesis (Jones, 1997). As a result, use of precisely controlled diets, through improved subject compliance, helps to improve the efficacy of plant sterols on hypocholesterolemia.

The reduction of LDL-C is differentiated by age in hypercholesterolemic individuals, with an average reduction of 0.54mmol/L in persons aged 50-59y, 0.43mmol/L in those aged 40-49y and 0.33mmol/L in those aged 30-39y (Law, 2000).

Type 2 diabetic individuals have a reduced absorption of cholesterol and up-regulated de novo synthesis. Therefore, it was observed that plant sterols have a lower efficacy in diabetics versus hypercholesterolemic individuals (Gylling and Miettinen, 1997; 1994).

Several mechanisms of cholesterol lowering action by plant sterols have been proposed. Both sitostanol and beta-sitosterol are thought to extrinsically and intrinsically reduce plasma cholesterol concentration (Jones et al., 1999). Extrinsically, plant sterols competitively block cholesterol absorption from the intestinal lumen (Heinemann et al., 1993; Heinemann et al., 1986), compete with intestinal brush border membrane for cholesterol uptake (Ikeda et al., 1988a; Ikeda et al., 1988b), displace cholesterol from bile salt micelles (Child et al., 1986), increase bile salt excretion (Salen et al., 1970), change micellar solubilization, intracellular cholesterol esterification rate and incorporation into chylomicrons (Child et al., 1986; Ikeda and Sugano, 1983). Reduced absorption is compensated for by enhanced synthesis of cholesterol (Jones et al., 2000, Miettinen et al., 2000). In addition, plant sterols may intrinsically modify hepatic acetyl-CoA carboxylase (Laraki et al., 1993) and cholesterol 7-α hydroxylase enzyme activities (Shefer et al., 1994) in animals and human.

Generally, plant sterol consumption in human subjects under a range of experimental circumstances reduces plasma total cholesterol and LDL-C concentrations within the range of 5-13% and 6-24%, respectively. In contrast to lowering total cholesterol and LDL-C, plant sterols did not exert a significant effect on HDL-C and TG in most studies (Jones et al., 1997). Jones et al. (2000) found that, after the intake of sterol and stanol ester containing diets for 3 weeks, cholesterol absorption was reduced by 36.2% and 25.9% respectively, compared to the control. Although cholesterol synthesis was increased in both diet groups, the investigators observed a 13.4% and 10.2% decrease in total cholesterol for sterol and stanol ester diets, respectively, and a 12.9% and 7.9%

decrease in LDL-C concentrations for sterol- and stanol-containing diets. The investigators also showed that the hydrogenation of the plant sterol ester mixture provided does not alter the suppression level of cholesterol in hypercholesterolemic individuals (Jones et al., 2000). In another study of hypercholesterolemic type 2 diabetic patients, 3g/d of plant sterols decreased VLDL-C by 12%, IDL-C by 11%, whereas HDL-C increased by 11% ($P<0.05$) (Gylling and Miettinen, 1994).

2.2.3. Effects of Plant Sterols on Plasma Fat Soluble Vitamins

Fat soluble vitamins, such as carotenoids, retinol, tocopherols, and vitamins D and K, are absorbed similarly to other lipids in the gut. Since plant sterol intake reduce intestinal cholesterol absorption, fat-soluble vitamins and their precursor absorption may be affected as well. However, current studies give inconsistent results.

Various studies have examined the relationship between plant sterol intake and carotenoid absorption. Weststrate and Meijer (1998) compared a sitostanol ester spread with esterified sterols from soybean, sheanut, or rice bran and found that all reduced lipid-standardized alpha- and beta-carotenoids to a variable extent (9-43%) and that the reduction was not related to the magnitude of lipid reduction. Similar results were reported with consumption of 3 different levels of plant sterol esters (0.85g/d, 1.61g/d, or 3.24g/d). However, after correcting for the reduction in plasma lipid levels, only plasma alpha- and beta-carotene levels were found to be reduced (Hendriks et al., 1999). Gylling et al. (1999) also reported reduction in plasma alpha- and beta-carotene, as well as alpha-tocopherol concentrations in subjects who consumed 2.5g sitostanol-ester-fortified

spread per day. Plat et al. (2001) also suggested that the most fat-soluble vitamins (beta-carotene, alpha-carotene, and lycopene) tended to be reduced as a result of reduction in LDL-C level compared with other less fat-soluble vitamins. In other studies that have shown a significant reduction on beta-carotene levels from plant sterol consumption, the subjects were consuming a higher fat intake (Gylling et al., 1996; Gylling and Miettinen, 1999; Weststrate and Meijer, 1998).

In contrast, Hallikainen et al. (1999) observed no significant change in lipid standardized alpha- and beta-carotene or lycopene levels with plant sterols consumed as part of a National Cholesterol Education Program Step 2 diet (fat intake was 26%, saturated fat 6.9% and cholesterol intake was 146 mg/d). More significantly, plant sterol intakes have not been shown to have any significant effect on another fat-soluble vitamin, retinal, for which beta-carotene is the precursor. Nor was there an effect on 25-OH vitamin D levels. Alpha-tocopherol levels were unchanged (Nguyen and Dale, 1999). In addition, Raeini-Sarjaz et al. (2002) reported no effect of sterol and stanol ester diets on serum retinol, alpha- and γ-tocopherol, vitamin D and K concentrations in male hypercholesterolemic subjects with precisely controlled diets. Christiansen et al. (2001) also showed that consuming plant sterols as part of a normal diet, did not cause statistically significant changes in serum retinol, α-tocopherol, ubiquinone, α-, or β-carotene concentrations. In conclusion, plant sterol consumption as part of a low fat and/ or a normal fat diet with increased intake of carotenoid-rich foods, such as fruits and vegetables, can negate the decrease in beta-carotene levels as well as other fat soluble vitamins that have been observed with plant sterol consumption.

2.2.4. Effects of Plant Sterols on Glycemic Control

Glycemic control can be evaluated by glycated hemoglobin (HbA1c). The glycation of hemoglobin reflects the concentration of glucose in the blood. The Canadian Diabetes Advisory Board considers HbA1c values below 115% of the upper limit of the reference value as optimal (Meltzer et al., 1998).

Nothing is known about the effects of plant sterols on glycemic control and there are very limited human studies examining the hypoglycemic effect of plant sterols. In a randomized, placebo-controlled, double-blind clinical trial over 12 weeks using plant sterol-enriched spread in subjects with type 2 diabetes, there was a small reduction in HbA1c compared to the control group which was only significant after 4 weeks (Lee et al., 2003). However, this study did not show any changes on fasting blood glucose as well as a more sensitive parameter, fructosamine. Therefore, it may require additional studies for verification.

3. RATIONALE

The functional role of plant sterols in the management of human hypercholesterolemic condition is well established. Due to hyperglycemia and hyperinsulinemia, individuals with type 2 diabetes have higher synthesis and lower absorption rates of cholesterol. However, whether the efficacy of plant sterols in diabetic subjects differs compared to that in non-diabetics has not been well established. Also, effects of plant sterols in altering the cholesterol absorption and synthesis have not been fully examined in type 2 diabetic subjects. As a result, further investigation is essential in defining dietary strategies for best normalizing risk of CHD and associated complications in type 2 diabetes. This study therefore attempted to characterize effects and efficacy of plant sterols on cholesterol levels and metabolism within type 2 diabetics.

4. HYPOTHESIS AND OBJECTIVES

4.1. HYPOTHESES

4.1.1 Ho: The plasma lipid profiles will be identical in hypercholesterolemic individuals with and without type 2 diabetes consuming controlled solid foods diets for 3 weeks.

4.1.2 Ho: There will be no effect of feeding plant sterols on plasma lipid profiles in hypercholesterolemic individuals with and without type 2 diabetes consuming controlled solid foods diets for 3 weeks.

4.1.3 Ho: There will be no effect of feeding plant sterols on glycemic control in hypercholesterolemic individuals with and without type 2 diabetes consuming controlled solid foods diets for 3 weeks.

4.2. OBJECTIVES

4.2.1 To compare plasma lipid profiles and glycemic control, in hypercholesterolemic individuals with and without type 2 diabetes consuming controlled solid foods diets for 3 weeks.

4.2.2 To examine the effect of feeding plant sterols on plasma lipid profiles and glycemic control, in hypercholesterolemic individuals with and without type 2 diabetes consuming controlled solid foods diets for 3 weeks.

5. MANUSCRIPT

Plant Sterols Are More Efficacious in Lowering Plasma Low Density and Non-high Density Lipoprotein Cholesterol in Hypercholesterolemic Type 2 Diabetics Versus Non-diabetic Individuals

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Running head: plant sterols, hypocholesterolemic agent, type 2 diabetes

6. ABSTRACT

Background: Due to hyperglycemia and hyperinsulinemia, type 2 diabetics have higher cholesterol synthesis and lower absorption rates. The difference in the efficacy of plant sterols (PS) in diabetic individuals compared to non-diabetic has not been previously examined.

Objective: To compare the degree of response of plasma lipid profiles and glycemic control between hypercholesterolemic type 2 diabetic and non-diabetic subjects to PS consumption in a controlled diet.

Design: Fifteen non-diabetic subjects (age = 55.1 ± 2.2 yr and BMI = 26.9 ± 0.8 kg/m^2) and fourteen diabetic subjects (age = 54.5 ± 1.8 yr and BMI = 30.2 ± 1.1 kg/m^2) participated in a double-blinded, randomized, crossover, placebo-controlled feeding trial. The diet included either 1.8g/d of PS or corn starch placebo each provided over 21 d separated by a 28 d washout period. Subjects consumed only foods prepared in Mary Emily Clinical Nutrition Research Unit of McGill University.

Results: Total cholesterol (TC) decreased ($p<0.05$) from baseline with PS for non-diabetic and diabetic subjects by 9.7% and 13.6% and decreased ($p<0.05$) with placebo for non-diabetic and diabetic subjects by 10.9% and 11.6%, respectively. Non-diabetic subjects had lower ($p<0.05$) mean endpoint LDL-cholesterol and non-HDL-cholesterol on PS diet (3.23 ± 0.25 mmol/L and 4.21 ± 0.15 mmol/L, respectively) than on placebo diet (3.51 ± 0.25 mmol/L and 4.34 ± 0.15 mmol/L, respectively). Diabetic subjects had lower ($p<0.05$) mean endpoint LDL-cholesterol and non-HDL-cholesterol on PS diet (2.34 ± 0.35 mmol/L and 3.69 ± 0.36 mmol/L, respectively) than on placebo diet (2.59 ± 0.35 mmol/L and 3.89 ± 0.36 mmol/L, respectively). The diabetic group displayed a

lower ($p<0.05$) mean endpoint LDL-cholesterol and non-HDL-cholesterol values from baseline value (day 0) on PS diet, as well as lower ($p<0.05$) mean endpoint LDL-cholesterol and non-HDL-cholesterol values than those of non-diabetic group on PS diet. **Conclusions:** Results demonstrated that PS is more efficacious in lowering LDL-cholesterol and non-HDL-cholesterol in diabetic individuals compared to non-diabetics. PS consumption may thus exist as a dietary management strategy for hypercholesterolemia in type 2 diabetics.

5.1 INTRODUCTION

Type 2 diabetes is often associated with metabolic disturbance including hyperinsulinemia, insulin resistance, frequent dyslipidemia, obesity and impaired beta-cell function (Ginsberg, 1991; Polonsky et al., 1996; Am. Diet. Assoc., 1999) and is frequently linked with several lipid aberrations, namely, hypertriacylglycerolemia, elevated very low density lipoprotein cholesterol (VLDL-C) and reduced HDL-C (Howard, 1987; De Fronzo et al., 1992; Howard and Howard, 1994; Laakso, 1996). However, elevated LDL-C levels are not uniformly present in dyslipidemic type 2 diabetic individuals (Harris, 1991). Studies have shown that individuals with type 2 diabetes have increased synthesis and decreased absorption of cholesterol (Briones et al., 1986; Gylling and Miettinen, 1994; Gylling et al., 1997; Strandberg et al., 1996; Simonen et al., 2000). As a result, the risk of developing atherosclerotic vascular diseases such as cardiovascular diseases (CVDs) is two- to seven-fold higher in type 2 diabetics compared to non-diabetic individuals (Stamler et al., 1993; Strandberg et al., 1996; Haffner et al., 1998).

Diabetic patients without a history of heart disease and non-diabetics with a history of myocardial infarction are equally at risk of suffering an infarction (Haffner et al., 1998). Thus, the target blood lipid levels are stricter for diabetic than non-diabetic individuals (Meltzer et al., 1998). The target values should, therefore, rest within the range of LDL-C at 2.5mmol/L or less; triacylglycerol (TAG) at 2.0mmol/L or less; and a TC/HDL-C ratio at 4.0 or less (Hallé, 2000).

Plant sterols (PS), chemically resembling cholesterol, have been shown to block absorption of dietary and endogenously-derived cholesterol from the gut (Jones et al.,

1997; Lichtenstein and Deckelbaum, 2001). PS are not synthesized by the human body and are minimally absorbed by the human intestine (Bouic, 1998). Daily consumption of 0.7-3.2g/d PS has been shown to reduce plasma TC by 5.0-13.0%, and LDL-C by 5.6-24.4% in both normo- and hypercholesterolemic individuals with (Gylling and Miettinen, 1994; Gylling and Miettinen, 1996; Law, 2000; Lee et al., 2003) and without type 2 diabetes (Miettinen et al., 1995; Jones et al., 1997; Jones and Ntanios, 1998; Jones et al., 1999; Hendriks et al., 1999; Pelletier et al., 1995, Weststrate and Meijer, 1998; Hallikainen et al., 2000; Jones et al., 2000; Law, 2000; Plat et al., 2000; Tammi et al., 2000; Nigon et al., 2001, Christiansen et al., 2001).

The efficacy of plant sterols in diabetic subjects compared to non-diabetics has not been well examined in previous studies. Further investigation is essential in defining dietary strategies for best normalizing risk of CVDs and associated complications in type 2 diabetes. Therefore, the objective of this study was to compare the degree of response of plasma lipid levels and glycemic control between hypercholesterolemic type 2 diabetic and non-diabetic subjects to PS consumption in a controlled diet.

5.2 SUBJECTS AND METHODS

Human subjects

Fourteen healthy but hypercholesterolemic, slightly overweight subjects with type 2 diabetes and fifteen hypercholesterolemic non-diabetic subjects were recruited from the surrounding community by advertisements in local newspapers and private and public medical clinics. The inclusion criteria for all subjects included ambulatory, aged between 40 to 80 years, and the presence of documented primary hypercholesterolemia with or without type 2 diabetes. Also, LDL-C were required to be in the range of 3 to 5 mmol/L, TAG less than 5 mmol/L, and body mass index (BMI) between 23 to 40 kg/m^2. Diagnostic criteria for type 2 diabetics include fasting plasma glucose level of $\geq$ 7 mmol/L and glycated hemoglobin (HbA1c) levels of 7-8%. Exclusion criteria included use of beta-blockers or diuretics, and personal history of CVD. Those reporting exercise at a frequency of $\geq$ 5 times per week, or ongoing pregnancy or lactation were excluded. Subjects were required to have refrained from using drug therapy for hypercholesterolemia for the 8-week period prior to the start of the study.

Prior to acceptance into study, subjects were required to undergo a complete physical examination. Fasting blood and urine samples were collected for serum biochemistry, hematology and urine analyses. Subjects were screened for chronic illnesses, including hepatic, renal, thyroid and cardiac dysfunction, prior to admission in the study.

Subjects received a thorough explanation of the study protocol and were given opportunities to discuss any queries with either the principal investigator, attending physician, or research coordinators before signing a consent form. The experimental

protocol was approved by the Human Ethical Review Committee of the Faculty of Agriculture and Environmental Sciences for the School of Dietetics and Human Nutrition at McGill University.

Study Protocol

The study was a randomized, double-blinded, crossover, placebo-controlled clinical trial using two 21-day dietary feeding periods, separated by a 28-day washout period. During the washout, subjects resumed their habitual diets without restriction (**Figure 1**).

At baseline (day 0) and end (day 21 and day 22) of each dietary phase, fasting blood samples were taken for the determination of circulating lipid levels. When comparing endpoints of the two dietary phases, the mean of day 21 and day 22 values was used in order to account for day-to-day variation in circulating cholesterol levels. Day 0 and 21 blood samples were also used for determination of circulating PS levels, insulin, and HbA1c levels. At the start (day 1) and end (day 21) of each dietary phase fasting blood samples were taken for determination of circulating fatty acids.

Subjects were given routine physical examinations at the beginning and end of each dietary phase by the attending physician. Throughout the trial, a physician familiar with the study protocol and diets was available, in case subjects experienced discomfort with the diet.

Experimental Diets

The baseline control diet (BCD) was planned based on Canada Food Guide to Healthy Eating and Good Healthy Eating Guide (Canadian Diabetes Association, 1990).

The BCD was comprised of solid foods, typical of those consumed in North America, and provided as three meals and one snack per day in a 3-day rotating menu (**Table 1**). The nutrient content of the BCD was calculated using Food Processor (Esha Research, Salem, OR, US), a computerized dietary analysis system. The BCD was designed to meet recommended levels of intake for all vitamins and minerals. The Mifflin equation was used to estimate individual subject basal energy requirements (Mifflin, 1990), which was then multiplied by an activity factor of 1.7 to compensate for the additional energy need of mildly to moderately active healthy adults (Shils et al, 1999). If subjects gained or lost weight during the first week of each dietary phase, adjustments were made to individual energy requirements to ensure that baseline body weights were maintained (Jones, 2000). Body weight was monitored daily before breakfast during feeding periods to assess change in body weight.

The BCD contained 55% carbohydrate in which at least 75% was complex carbohydrate, with low glycemic index food selected where possible. The polyunsaturated:monounsaturated:saturated fatty acid ratio of the 30% of energy provided as fat was maintained at 1:1:1. Safflower, canola and flaxseed oil were used to provide most of the PUFA, while extra virgin olive oil was selected to provide MUFA. Saturated fat came from fats in the meat and palm oil. Flaxseed and canola oil were used as sources of essential fatty acids at the level of 2 to 3 %. Protein comprised 15% of ingested energy.

During each feeding period, a total of 1.8g/d of either plant sterol (PS phase) powder or placebo phase corn starch was added to margarine and served on the breakfast toast every morning under supervision. The PS powder was unesterified plant sterols

extracted from wood pulp byproducts (40% sitosterol, 30% campesterol, 20% dihydrobrassicasterol, 10% others). The placebo powder was corn starch, as it strongly resembled the white powdery PS (Vanstone et al., 2002). To achieve double blinding, PS powder and the corn starch were portioned in coded containers by an external party so that neither the researchers, nor the subjects, knew its true identity. Diets were prepared in the metabolic kitchen of the Mary Emily Clinical Nutrition Research Unit of McGill University. All subjects were required to consume breakfast at the unit under supervision and the other two meals and one snack were available for take out. No extra food was allowed between meals, except for decaffeinated, energy-free carbonated beverages and herbal teas, which were obtained from the kitchen's staff. Alcoholic beverages and coffee were prohibited during dietary phases.

Analyses

Plasma Lipid concentrations

Blood samples were drawn after a 12-hour overnight fast, and at least 24 hours of alcoholic abstinence (day 0), collected in EDTA-containing Vacutainer® (BD, Franklin Lakes, NJ, US) tubes. Samples were immediately centrifuged, and resulting plasma and red blood cell subfractions separated within an hour of collection, and stored at -80°C until analysis. Plasma TC, HDL-C and TAG, were analyzed in quadruplicate with standardized reagents using a VP Autoanalyser (Abbott Laboratories, North Chicago, IL, US). Calibration of the machine prior to each run was performed as per the standardization protocol of the Canadian Reference Laboratory (1996, Vancouver, BC, Canada), which involved direct comparison with fresh specimen samples. Measurement

of HDL-C in plasma was done after precipitation of apolipoprotein B with dextran sulfate and magnesium chloride (Warnick et al., 1982). LDL-C concentrations were calculated using the Friedewald equation (Friedewald et al., 1972).

Fatty acid methyl ester composition

Red blood cells (RBC) on day 1 and day 21 were analyzed in duplicate for fatty acid composition by gas-liquid chromatography (GLC) (HP 5890 Series II, Palo Alto, CA). A modified Folch extraction was used to extract total lipids from the samples (Folch et al., 1957) and the fatty acids methylated as per the procedure by Al Makdessi et al. (1985). Packed RBC and C17 standard (1mg/mL) were placed into a culture tube and MeOH was added to the sample. The culture tube was then heated to 55°C in a water bath for 15 minutes. A solution of hexane:chloroform (4:1 v/v) was added and placed in a wrist action shaker for 15 minutes. Millipore water was added and the sample shaken for an additional 10 minutes. The sample was centrifuged at 1500 rpm at 4°C for 15 minutes and the organic supernatant phase transferred to culture tube and dried under N_2 at 45°C. The aqueous layer was then re-extracted by adding hexane:chloroform and shaking the sample for 15 minutes. It was centrifuged at 1500 rpm and the supernatant added to the first extraction and dried down. Methylating reagent (7:6:7 BF_3MeOH:benzene:MeOH) was added to the sample. The tube was flushed with N_2, sealed with Teflon tape and vortexed. The tubes were heated at 100°C for 55 minutes and allowed to cool in tepid water. Hexane and millipore water were added to the sample, after which it was vortexed. The top layer was transferred to a 1.5 ml crimp seal vial dried down under N_2 and chloroform added and transferred to polypropylene vial inserts.

The composition of fatty acid methyl esters of RBC was determined using a Hewlett-Packard 5890 GLC, equipped with a 30 m x 0.2 mm SP 2330 column (Supelco, Bellefonte, PA), flame ionization detectors and automated injection (Howell et al., 1998). Briefly, the oven temperature was held at 100°C for 1 minute and increased to 190°C at a rate of 3°C/minute, after which it was held at this temperature for the remainder of the run. The injector temperature was set at 210°C and the detector temperature at 250°C. Fatty acid methyl esters were identified based on the retention time of known standards (Supelco, Belfonte, PA). All results were expressed as percent of total fatty acids by weight (wt/wt%).

Plasma plant sterol levels determination

Plant sterols were measured by GLC (HP 5890 Series II, Palo Alto, CA) facilitated with flame ionization detection and auto-injector system as described (Jones et al., 1999; Ntanios et al., 1999 and Jones et al., 2000). A 30 m SAC-5 column (Sigma-Aldrich Canada Ltd., Oakville, Ont.) was used. Briefly, an internal standard, 5 alpha-cholestane, was added to each plasma sample. Samples were saponified and sterols were extracted, re-suspended in chloroform, and injected into the GLC. The column temperature was 285°C. Isothermal running conditions were maintained for 42 minutes. The injector and detector were set at 300°C and 310°C, respectively. The carrier gas (helium) flow rate was 1.2 ml/min and inlet splitter set at 100:1. Plant sterols were identified compared to authentic standards (Sigma-Aldrich Canada Ltd., Oakville, Ont.). Internal standards were used to calculate detector response factors.

Glycemic control determination

Insulin level measurements were performed on day 0 and day 21 plasma samples in duplicate using commercially available radioimmunoassay kits (ICN Pharmaceuticals, INC, Costa Mesa, CA) as ^{125}I as a tracer. Radioactivity was determined by gamma counting (LKB Wallac, 1282 compugamma CS. Fisher Scientific Montreal Canada) and collected as counts per min (CPM). Plasma values were quantified using a standard curve and automated data reduction procedures. Insulin values were expressed as μU/mL.

HbA1c on day 0 and day 21 blood samples were analyzed at a clinical diagnostic laboratory (LDS, Montreal, Qc.).

Statistics

All data were expressed as the mean ± standard error mean (SEM). Percentage changes from day 0 to day 21 and endpoint comparison (day 21/day 22) of plasma lipid profiles all used a crossover design (Proc mixed, SAS Version 8.0) for the determination of the time effect, treatment effect and interactions ($p<0.05$). Student's paired t-tests were used to compare baseline values (day 0) with final time points (day 21) of plasma plant sterols, insulin and HbA1c levels. RBC fatty acid composition in the beginning (day 1) and at the end (day 21) of each dietary phase were compared using student's paired t-tests to show the change within each phase. A level of statistical significance at $p<0.05$ was used in all analyses.

5.3 RESULTS

Subject compliance and drop-out rate

Sixteen hypercholesterolemic non-diabetics and sixteen diabetics were enrolled into the study. One non-diabetic subject dropped out at the first week of the second feeding cycle because of a myocardial infarction. Two diabetic subjects dropped out at the first week of the first feeding cycle due to personal reasons. Therefore, complete data from fifteen non-diabetics and fourteen diabetic subjects were collected and analyzed as per the study protocol. The BCD was overall well tolerated, except for minor gastrointestinal discomfort, which did not require medical intervention or lead to any subject withdrawing from the study protocol. During the first week of the first feeding trial, some subjects reported that they were given too much food, due to larger meal size compared to their habitual diets. Subjects consumed all food provided. However, compliance could not be confirmed because subjects took most of their meals home.

Subject characteristics

Baseline characteristics of the study subjects are presented in **Table 2**. Fifteen non-diabetic subjects (mean age = 55.1 ± 2.2 yr and mean BMI = 26.9 ± 0.8 kg/m^2) and fourteen diabetic subjects (mean age = 54.5 ± 1.8 yr and mean BMI = 30.2 ± 1.0 kg/m^2) participated. There were an equal number of female subjects (n = 9) in both groups but there were more male subjects (n = 6) in non-diabetic group compared to diabetic group (n = 5). The mean age (55.1 ± 2.2 yr), weight (76.6 ± 2.6 kg), TC (6.12 ± 0.21 mmol/L), LDL-C (3.95 ± 0.17 mmol/L) and HDL-C (1.39 ± 0.09 mmol/L) of non-diabetic subjects were not significantly different from diabetic subjects (age = 54.5±6.7 yr, weight = 82.8

± 2.44 kg, TC = 6.21 ±0.19 mmol/L, LDL-C = 4.24 ± 0.46 mmol/L and HDL-C = 1.22 ± 0.07 mmol/L). However, non-diabetic subjects had lower ($p<0.05$) BMI (26.9 ± 0.8 kg/m^2), TAG (1.71 ± 0.18 mmol/L), fasting blood glucose (FBG) (4.83 ± 0.09 mmol/L) and HbA1c (0.055 ± 0.002% of total hemoglobin) values, compared with diabetic subjects (BMI = 30.2 ± 1.1 kg/m^2, TAG = 3.00 ± 0.36 mmol/L, FBG = 9.66 ± 0.79 mmol/L and HbA1c = 0.074 ± 0.004 % of total hemoglobin). There was no weight change in either phase for either group (data not shown).

Plasma lipid profiles in response to treatment

The concentrations of plasma lipids at baseline (day 0) and end (day 21) of each dietary phase are shown in **Table 3.** TC decreased ($p<0.05$) from baseline with PS for non-diabetic and diabetic subjects by 9.7% and 13.6%, respectively. TC decreased ($p<0.05$) from baseline with placebo for non-diabetic and diabetic subjects by 10.9% and 11.6%, respectively. Non-HDL-C decreased ($p<0.05$) from baseline with PS for diabetic subjects by 18.5%. LDL-C levels were reduced ($p<0.05$) from baseline with PS for non-diabetic and diabetic subjects by 14.9% and 29.8%, respectively. There were no significant changes in HDL-C and TAG across diets or treatments.

Percent change in TC from baseline showed large between-subject variability. **Figure 2** shows the individual data for plasma TC between endpoints (average of day 21 and day 22) of each diet for non-diabetics and diabetic subjects. For non-diabetic subjects, the mean endpoint (average of day 21 and day 22) TC concentration on PS diet (5.45 ± 0.17 mmol/L) was not significantly different from mean endpoint value on

placebo diet (5.53 ± 0.15 mmol/L) and the percent changes of two phases were not significantly different, -9.74% on PS diet and -10.87% on placebo diet. For diabetic subjects, the mean endpoint TC concentration on PS diet (5.00 ± 0.32 mmol/L) was not different from mean endpoint value on placebo diet (5.03 ± 0.32 mmol/L) and the percent changes of two phases were not significant, -13.65% on PS diet and -11.63% on placebo diet. **Figure 3** shows the individual subject variation in plasma LDL-C between endpoints (average of day 21 and day 22) of each diet for non-diabetic and diabetic subjects. For non-diabetic subjects (n = 15), the mean endpoint LDL-C concentration on PS diet (3.23 ± 0.25 mmol/L) was lower ($p<0.05$) than mean endpoint LDL-C value on placebo diet (3.51 ± 0.25 mmol/L). For diabetic subjects (n = 13), the mean endpoint LDL-C concentration on PS diet (2.34 ± 0.35 mmol/L) was lower ($p<0.05$) than mean endpoint LDL-C value on placebo diet (2.59 ± 0.35 mmol/L). Both non-diabetic and diabetic groups had lower ($p<0.05$) mean endpoint LDL-C values from baseline values (day 0) on PS diet. The diabetic group had lower ($p<0.05$) mean endpoint LDL-C values compared with the non-diabetic group on PS diet. **Figure 4** shows the individual subject variation in plasma non-HDL-C between endpoints of each diet for non-diabetic and diabetic subjects. For non-diabetic subjects (n = 15), the mean endpoint non-HDL-C concentration on PS diet (4.21 ± 0.15 mmol/L) was lower ($p<0.05$) than mean endpoint non-HDL-C value on placebo diet (4.34 ± 0.15 mmol/L). For diabetic subjects (n = 14), the mean endpoint non-HDL-C concentration on PS diet (3.69 ± 0.36 mmol/L) was lower ($p<0.05$) than mean endpoint non-HDL-C value on placebo diet (3.89 ± 0.36 mmol/L). The diabetic group displayed a lower ($p<0.05$) mean endpoint non-HDL-C

value from baseline value (day 0) on PS diet, as well as a lower ($p<0.05$) mean endpoint non-HDL-C value than that of non-diabetic group on PS diet.

Plasma plant sterol levels in response to treatment

Plasma PS concentrations and ratios relative to TC are presented in **Table 4**. Plasma campesterol and beta-sitosterol concentrations were not different between groups at the baseline or the end of each dietary phase. Moreover, there were no changes between groups at the baseline and the end of each dietary phase for campesterol:beta-sitosterol ratio, campesterol:total cholesterol ratio, beta-sitosterol:total cholesterol ratio. However, after consumption of the PS diet, non-diabetic group revealed higher ($p<0.05$) campesterol:beta-sitosterol ratio than diabetic group (+316.2% vs. +9.3%, respectively).

Red blood cell fatty acid composition within each phase

Relative fatty acid composition data in red blood cells (RBC) on day 1 and day 21 of each dietary phase for both groups are presented in **Table 5**. Day 1 and day 21 values were compared to see the change in fatty acid composition within each dietary phase. Overall, there was no significant change in fatty acid composition for either group during placebo diet phase. Generally, fatty acid composition did not change significantly in non-diabetic subjects on PS diet, except that the ratio of C18:0 increased by 37.0% and the sum of monounsaturated fatty acids decreased by 10.5% relative to day 1 values. There were no significant changed observed in fatty acid composition for diabetic subjects except C16:0 decreased by 13.8% and C18:0 increased by 31.7% relative to day 1 of the PS diet phase.

Glycemic control in response to treatment

There was no significant change in plasma insulin levels for both groups after consumption of each diet for 21 days (**Figure 5**). Plasma insulin changes between the two groups were not statistically different with and without PS consumption.

Values for HbA1c levels are shown in **Figure 6**. There was no significant change in HbA1c levels for either groups after consumption of each diet for 21 days (**Figure 6**). There was no improvement in HbA1c for non-diabetic subjects on PS diet or the placebo diet (0% and -0.3%, respectively). However, HbA1c tended to improve for diabetic subjects on PS diet and placebo diet (-2.3% and -3.7%, respectively), however the improvement was not statistically significant.

Associations between plasma lipid levels and plasma plant sterol levels

Across all subjects, both plasma TC ($r = 0.30$, $p = 0.0017$) and LDL-C ($r = 0.38$, $p = 0.0001$) levels varied directly with plasma beta-sitosterol concentration. However, neither plasma TC nor LDL-C were found to be associated with plasma campesterol and campesterol:beta-sitosterol ratio.

5.4 DISCUSSION

The present findings show that PS consumption yields a larger reduction of LDL-C and non-HDL-C were greater in type 2 diabetic individuals compared with non-diabetic subjects. This effect may be perhaps due to a relatively low absorption efficiency (Briones et al., 1986) as part of the insulin resistance syndrome (Simonen et al., 2000) of type 2 diabetics. In general, the results of this study are in good agreement with earlier studies of various designs using PS, which showed TC and LDL-C reduction in the range of 5.0-13.0% and 5.6-24.4%, respectively (Gylling and Miettinen, 1994; Miettinen et al., 1995; Gylling and Miettinen, 1996; Jones et al., 1997; Weststrate and Meijer, 1998; Jones and Ntanios, 1998; Jones et al., 1999; Jones et al., 2000; Hallikainen et al., 2000; Plat et al., 2000; Law, 2000; Nigon et al., 2001; Christiansen et al., 2001; Lee et al., 2003). In the present study, TC decreased by 13.7% and LDL-C by 29.9% after 21 days versus baseline for diabetic subjects. These reductions are above the range from previous studies. On the other hand, non-diabetic subjects had a 9.7% reduction in TC and 14.9% reduction in LDL-C, which are within the previously reported reduction range.

The decrease in TC did not differ between PS and placebo phases across groups. This result, which was not consistent with most other studies examining PS efficacy, was perhaps due to several features of study design, including the plasma cholesterol modifying characteristics of the control diet, subject-specific type of lipid disorder, or PS dose and composition (Jones et al., 1997). The BCD used in this study were comprised of 55% carbohydrate, in which complex carbohydrate made up about 75%. Results of NHANES III showed that moderately high carbohydrate (50% to 55% of energy) diets

were associated with low CVD risks with favorable lipid profiles (Yang et al., 2003). In this study, both non-diabetic and diabetic subjects had hypercholesterolemia, but diabetic subjects also had hypertriacylglycerolmia (**Table 2**). Unesterified PS powder (40% sitosterol, 30% campesterol, 20% dihydrobrassicasterol, 10% others) was mixed in margarine and added to the controlled diet. Although Vanstone et al. (2002) showed that both plant sterols and stanols, in their unesterified form, significantly and equally reduced both plasma TC and LDL-C concentrations, the specific composition of unesterified PS powder used in this study differed from those used in previous studies. Moreover, BCD used in this study was different from those used in previous studies. Thus, the degree of cholesterol lowering observed might also be expected to be different.

In agreement with earlier studies, plant sterols did not affect plasma HDL-C and TAG concentrations significantly (Miettinen et al, 1995; Weststrate and Meijer, 1998; Hendriks et al., 1999; Hallikainen and Uusitupa, 1999; Jones et al., 2000; Jones et al., 1999). On the other hand, present results demonstrate for the first time that PS consumption reduces non-HDL-C in hypercholesterolemic diabetic subjects. Cui et al. (2001) indicated that non-HDL-C level was a stronger and better predictor of CVD mortality than LDL-C level in men and women without clinical evidence of CVD. In addition to the reduction in LDL-C, this study showed that PS consumption in a controlled diet for 21 days substantially lowered non-HDL-C in diabetic group (18.6%, $p<0.05$). As a result, the risk of CVD mortality for the people with type 2 diabetes consuming PS along with a heart healthy diet might feasibly be reduced.

The red blood cell fatty acid compositions reflect dietary habits (Korpela et al., 1999; Schwab, 1998). There were no differences in red cell fatty acid composition from

day 1 to day 21 within two dietary phases, except C16:0 decreased and C18:0 increased for diabetic group. These data provided indirect evidence that there was good compliance with the dietary modification because an absence in changes in RBC fatty acid composition should be taken as reflecting no change in the diet within any of dietary phases. Moreover, given that all diets in this study were isocaloric, there were no statistically significant changes in the body weight for both groups after 2 dietary phases (data not shown). These data provide additional indirect evidence that there was good compliance of the subjects with the diets.

Plasma PS concentrations have been used as an indirect measure of cholesterol absorption. Specifically, serum campesterol concentration and the campesterol:cholesterol ratio have been shown to correlate positively with intestinal cholesterol absorption. With controlled diets, this association would be expected to reflect cholesterol absorption. However, various PS are absorbed and metabolized differently. Thus, it is not appropriate to use plasma campesterol to measure cholesterol absorption (Vanstone et al., 2002). Although plasma PS levels increased after the PS phase, the change did not reach statistical significance. Sitosterol makes up 40% of the PS regimen used in this study. Previous studies have shown that plasma campesterol and sitosterol levels increase (Jones et al., 2000) or remain unchanged (Becker et al., 1993) with sitosterol feeding. Given that PS consumption varied across the dietary phases, use of campesterol or sitosterol as a marker of cholesterol absorption would be highly inappropriate. In the present study, plasma sterol levels increased, but not significantly, with sterol feeding, which is in agreement with previous work (Vanstone et al., 2002).

Another objective of this study was to examine possible effects of PS consumption on glycemic control, since little is known about this issue. There was no improvement in either HbA1c nor insulin with PS feeding. This result was in disagreement with recent work of Lee et al. (2003) in which the reduction of HbA1c in type 2 diabetic subjects, under free-living conditions, was significant after using a plant sterol-enriched spread for 4 weeks. The difference in improvement between the two studies may be due to the duration of the studies and the degree of glycemic control of type 2 diabetic subjects before they enrolled in the studies.

Using non-HDL-C to estimate the CVD mortality risk overcomes the limitation of using Friedewald equation to calculate LDL-C levels. Because of the ease of measuring non-HDL-C levels by a simple subtraction of HDL-C level from TC levels, and because of invalid LDL-C calculation with TAG level > 4.5 mmol/L using the Friedewald equation. However, a few diabetic subjects in this study had TAG > 4.5 mmol/L. As a result, their LDL-C levels were not included in the results.

In summary, the present study demonstrates for the first time that consumption of PS is more efficacious in lowering LDL-C and non-HDL-C in type 2 diabetics versus non-diabetic individuals. PS therefore serves as a potential adjunct to dietary management of hypercholesterolemia in type 2 diabetics. As the risk of developing CVD is two- to seven-fold higher in type 2 diabetics compared to non-diabetic individuals, this study demonstrates that PS consumption improves the excessive cardiovascular risk in this population. Therefore, incorporation of PS into the low saturated fat and low cholesterol diet of individuals at increased CVD mortality risk could have a positive impact on reducing the mortality rate of type 2 diabetes.

TABLE 1

Cycle menu of the baseline control diet

Meal	Day 1	Day 2	Day 3
Breakfast	Orange juice	Apple juice	Orange juice
	2% milk	2% milk	2% milk
	Oatmeal w/ brown sugar	Cereal	Croissant
	Whole wheat toast	Whole wheat toast	Margarine
	Margarine	Margarine	
	0.1% Yogurt		
	Peach		
Snack	Pear	Apple	Apple sauce
	Apple juice	Orange juice	Grape juice
	Bagel	Homemade chocolate	
	cream cheese	pudding	
Lunch	Chicken soup	Chicken fingers	Cream of
	Ham and cheese sandwich	Rice	vegetable
	with	Peas & carrots	Macaroni &
	Whole wheat bread and	2% milk	cheese
	lettuce	Applesauce	Apple
	2% milk		0.1% Yogurt
	Fruit salad		
Dinner	Spaghetti	Chicken soup	Tomato lentil soup
	Homemade tomato spaghetti	Whole wheat toast	Filet of sole
	sauce	2% milk	Carrots
	Vegetables	Beef stew	Brown rice
	2% milk	Grapes	2% milk
	Pear		Fruit salad
	Carrot cake		Date square

TABLE 2

Baseline characteristics of subjects[1]

	Non-diabetics (n = 15)	Type 2 Diabetics (n = 14)
Gender	Female = 9 Male = 6	Female = 9 Male = 5
Age (year)	55.1 ± 2.2	54.5 ± 1.8
Weight (kg)	76.6 ± 2.6	82.8 ± 2.4
BMI (kg/m^2)[2]	26.9 ± 0.8[a]	30.2 ± 1.1[b]
Total cholesterol (mmol/L)	6.12 ± 0.21	6.11 ± 0.17
LDL cholesterol (mmol/L)[3]	3.95 ± 0.17	3.62 ± 0.14
HDL cholesterol (mmol/L)[4]	1.39 ± 0.09	1.22 ± 0.07
Triacylglycerol (mmol/L)	1.71 ± 0.18[a]	3.00 ± 0.36[b]
Fasting glucose (mmol/L)	4.83 ± 0.09[a]	9.66 ± 0.79[b]
HbA1c[5] (% of total hemoglobin)	0.055 ± 0.002[a]	0.073 ± 0.004[b]

[1]Values are expressed as mean ± SEM
[2]Body mass index
[3]Low-density lipoprotein cholesterol
[4]High density lipoprotein cholesterol
[5]Glycated hemoglobin
Values carrying different superscript letters across a row indicate significant difference between two groups ($p<0.05$)

TABLE 3

Plasma lipid levels at day 0 and day 21 of each dietary phase[1]

Lipid	Plant Sterol		Placebo	
	Non-diabetics	Diabetics	Non-diabetics	Diabetics
Total cholesterol (mmol/L)	n= 15	n = 14	n = 15	n = 14
Day 0	6.06 ± 0.24	5.84 ± 0.39	6.21 ± 0.25	5.55 ± 0.32
Day 21	5.39 ± 0.18*	4.89 ± 0.29*	5.49 ± 0.14*	4.94 ± 0.35*
% Change	-9.74 ± 4.52	-13.65 ± 4.59	-10.87 ± 4.52	-11.63 ± 4.59
LDL cholesterol (mmol/L)[2]	n = 14	n = 12	n = 13	n = 14
Day 0	3.92 ± 0.27	3.24 ± 0.35	3.84 ± 0.29	2.95 ± 0.31
Day 21	3.29 ± 0.23*	2.48 ± 0.33*	3.59 ± 0.20	2.67 ± 0.35
% Change	-14.87 ± 7.11[a]	-29.83 ± 7.26[b]	-3.31 ± 7.25[c]	-5.97 ± 7.12[c]
HDL cholesterol (mmol/L)[3]	n = 15	n = 14	n = 15	n = 14
Day 0	1.32 ± 0.10	1.37 ± 0.13	1.34 ± 0.10	1.18 ± 0.07
Day 21	1.23 ± 0.11	1.37 ± 0.18	1.22 ± 0.09	1.08 ± 0.08
% Change	-8.12 ± 6.68[a]	+7.34 ± 6.90[b]	-7.81± 6.68[a]	-8.81± 6.90[a]
Non-HDL cholesterol (mmol/L)[4]	n = 15	n = 14	n = 15	n = 14
Day 0	4.74± 0.22	4.47 ± 0.41	4.87 ± 0.21	4.37 ± 0.34
Day 21	4.16 ± 0.16	3.51 ± 0.34*	4.28 ± 0.15	3.87 ± 0.35
% Change	-7.40 ± 7.23	-18.55± 7.31	-12.07 ± 7.23	-12.60 ± 7.31
Triacylglycerol (mmol/L)	n = 15	n = 14	n = 15	n = 14
Day 0	1.98 ± 0.31	2.71 ± 0.53	2.26 ± 0.27	3.12 ± 0.47
Day 21	2.07 ± 0.34	2.75 ± 0.49	1.97 ± 0.40	2.63 ± 0.42
% Change	+11.07 ± 11.92[a]	+11.25 ± 12.11[a]	-9.60 ± 11.92[b]	-13.86 ± 12.11[b]

[1]Values are expressed as mean ± SEM

[2]Low-density lipoprotein cholesterol

[3]High density lipoprotein cholesterol

[4]Non-high density lipoprotein cholesterol = total cholesterol – high density lipoprotein cholesterol

*Significantly different from day 1 within dietary phase ($p<0.05$)

Values carrying different superscript letters across a row indicate treatment effect ($p<0.05$)

TABLE 4

Plasma plant sterol levels at day 0 and day 21 of each dietary phase[1]

Plant Sterol	**Plant Sterol**		**Placebo**	
Parameters	Non-diabetics	Diabetics	Non-diabetics	Diabetics
Campesterol (μmol/L)	n = 15	n = 14	n = 15	n = 14
Day 0	5.6 ± 1.2	6.8 ± 1.7	6.3 ± 2.1	6.7 ± 1.6
Day 21	6.0 ± 1.2	7.5 ± 1.8	7.2 ± 1.6	4.5 ± 0.9
% Change	+25.4 ± 14.4	+34.8 ± 16.0	+35.3 ± 15.7	+4.1 ± 21.4
Beta-sitosterol (μmol/L)	n = 15	n = 13	n = 13	n = 11
Day 0	1.4 ± 0.5	1.5 ± 0.6	2.2 ± 0.8	1.1 ± 0.4
Day 21	1.2 ± 0.4	1.6 ± 0.4	1.4 ± 0.3	1.2 ± 0.5
% Change	+18.5 ± 45.9	+111.7 ± 66.4	+49.1 ± 42.9	+12.3 ± 23.4
Campesterol:Beta-sitosterol	n = 14	n = 12	n = 15	n = 12
Day 0	10.0 ± 4.4	14.0 ± 6.3	11.3 ± 5.0	23.7 ± 14.9
Day 21	18.8 ± 5.2	10.8 ± 5.5	11.8 ± 4.1	29.2 ± 19.7
% Change	+316.2 ± 129.4[a]	+9.34 ± 31.9[b]	+1085.5± 1011.9	+91.9 3± 58.3
Campesterol:TC [2]	n = 15	n = 14	n = 15	n = 14
Day 0	2.3± 0.7	2.8 ± 0.7	2.5 ± 1.0	3.1 ± 1.0
Day 21	2.6 ± 0.8	4.0 ± 1.4	3.2 ± 1.0	1.7 ± 0.3
% Change	+34.4 ± 16.2	+50.3 ± 17.5	+68.4 ± 31.3	+9.4 ± 22.7
Beta-sitosterol:TC	n = 15	n = 13	n = 15	n = 14
Day 0	5.6 ± 1.2	0.6 ± 0.2	0.9 ± 0.3	0.4 ± 0.1
Day 21	6.0 ± 1.2	0.7 ± 0.2	0.6 ± 0.2	0.4 ± 0.2
% Change	+133.3 ± 117.3	+201.9 ± 94.1	+453.0 ± 391.4	+196.4 ± 199.2

[1]Values are expressed as mean ± SEM

[2]Total cholesterol

Values carrying different superscript letters across a row indicate significant difference between two groups within diets ($p<0.05$)

TABLE 5

Fatty acid composition of red blood cells at the beginning and the end of each dietary phase[1]

Fatty acid	Group	Plant Sterol		Placebo	
% of total identified fatty acids		Day 1	Day 21	Day 1	Day 21
14:0	Non-diabetics	0.49 ± 0.07	0.52 ± 0.07	0.49 ± 0.07	0.52 ± 0.07
	Diabetics	0.40 ± 0.05	0.60 ± 0.12	0.54 ± 0.13	0.44 ± 0.08
16:0	Non-diabetics	33.92 ± 1.07	32.14 ± 1.05	33.04 ± 1.28	31.83 ± 0.86
	Diabetics #	35.32 ± 1.01	30.46 ± 2.06*	33.50 ± 1.00	33.65 ± 0.89
18:0	Non-diabetics	11.64 ± 1.55	15.95 ± 1.53*	12.96 ± 1.51	15.04 ± 1.24
	Diabetics	12.75 ± 1.03	16.79 ± 1.54*	13.48 ± 1.12	15.89 ± 1.07
18:1n-9	Non-diabetics #	21.59 ± 0.96	19.39 ± 0.93	20.75 ± 0.94	21.44 ± 1.11
	Diabetics	21.21 ± 1.07	19.58 ± 1.17	19.85 ± 1.07	19.54 ± 0.95
18:2n-6	Non-diabetics	23.43 ± 1.44	22.08 ± 1.72	23.02 ± 1.92	21.82 ±1.43
	Diabetics	21.75 ± 1.86	23.07 ± 1.77	25.86 ± 1.84	22.34 ± 1.70
18:3n-3	Non-diabetics	0.62 ± 0.15	0.60 ± 0.10	0.87 ± 0.14	0.87 ± 0.21
	Diabetics	0.70 ± 0.13	0.66 ± 0.18	0.53 ± 0.10	0.50 ± 0.11
20:4n-6	Non-diabetics	4.27 ± 0.83	4.44 ± 0.90	4.94 ± 1.02	4.22 ± 0.80
	Diabetics	4.16 ± 0.94	5.27 ± 1.20	2.90 ± 0.68	4.20 ± 0.96
20:5n-3	Non-diabetics	0.67 ± 0.17	0.86 ± 0.25	0.83 ± 0.17	0.77 ± 0.20
	Diabetics	0.73 ± 0.14	0.63 ± 0.15	0.49 ± 0.15	0.43 ± 0.11
22:4n-6	Non-diabetics	0.82 ± 0.23	1.08 ± 0.28	0.81 ± 0.23	0.82 ± 0.20
	Diabetics	1.01 ± 0.24	1.05 ± 0.24	0.92 ± 0.34	1.01 ± 0.25
22:6n-3	Non-diabetics	1.65 ± 0.42	2.25 ± 0.49	1.31 ± 0.27	1.83 ± 0.36
	Diabetics	1.79 ± 0.39	1.35 ± 0.40	1.24 ± 0.36	1.55 ± 0.36
ΣSFA	Non-diabetics	46.05 ± 1.15	48.60 ± 1.89	46.55 ± 1.15	47.42 ± 1.78
	Diabetics	48.46 ± 1.65	47.85 ± 1.45	47.52 ± 1.56	49.97 ± 1.57
ΣMUFA	Non-diabetics #	22.48 ± 0.81	20.13 ± 0.88*	21.63 ± 0.81	22.25 ± 1.06
	Diabetics	21.63 ± 1.03	20.15 ± 1.14	20.59 ± 0.98	20.16 ± 0.90
Σn-6 PUFA	Non-diabetics	28.52 ± 0.76	27.60 ± 1.02	28.76 ± 1.11	26.86 ± 1.22
	Diabetics #	26.92 ± 1.16	29.39 ± 1.40	29.68 ± 1.36	27.48 ± 1.06
Σn-3 PUFA	Non-diabetics	2.93 ± 0.60	3.71 ± 0.71	3.01 ± 0.31	3.47 ± 0.64
	Diabetics	2.99 ± 0.53	2.65 ± 0.62	2.13 ± 0.50	2.34 ± 0.42

[1]Values are expressed as mean ± SEM

*Day 21 value was significantly different from day 1 value within dietary phase ($p<0.05$)

#Diet effect ($p<0.05$)

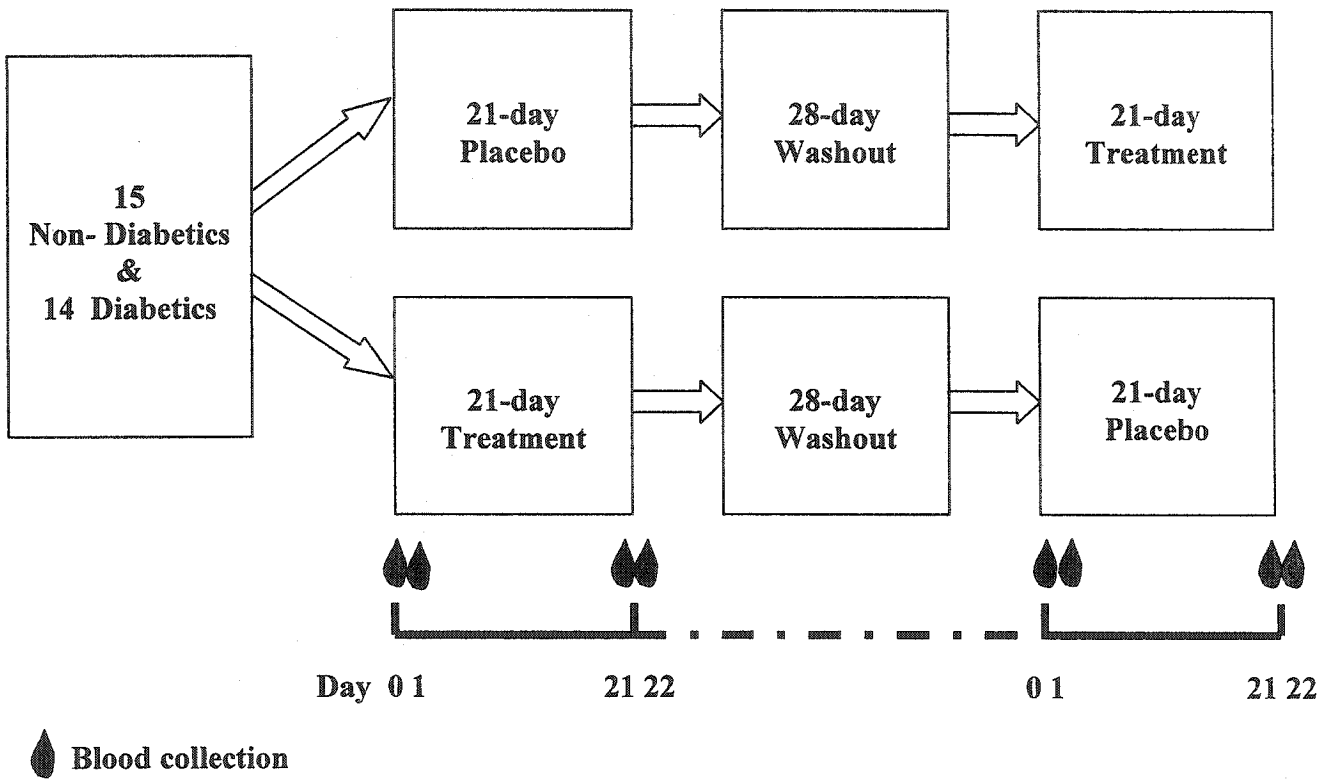

FIGURE 1 Study Design and time-line used in the study

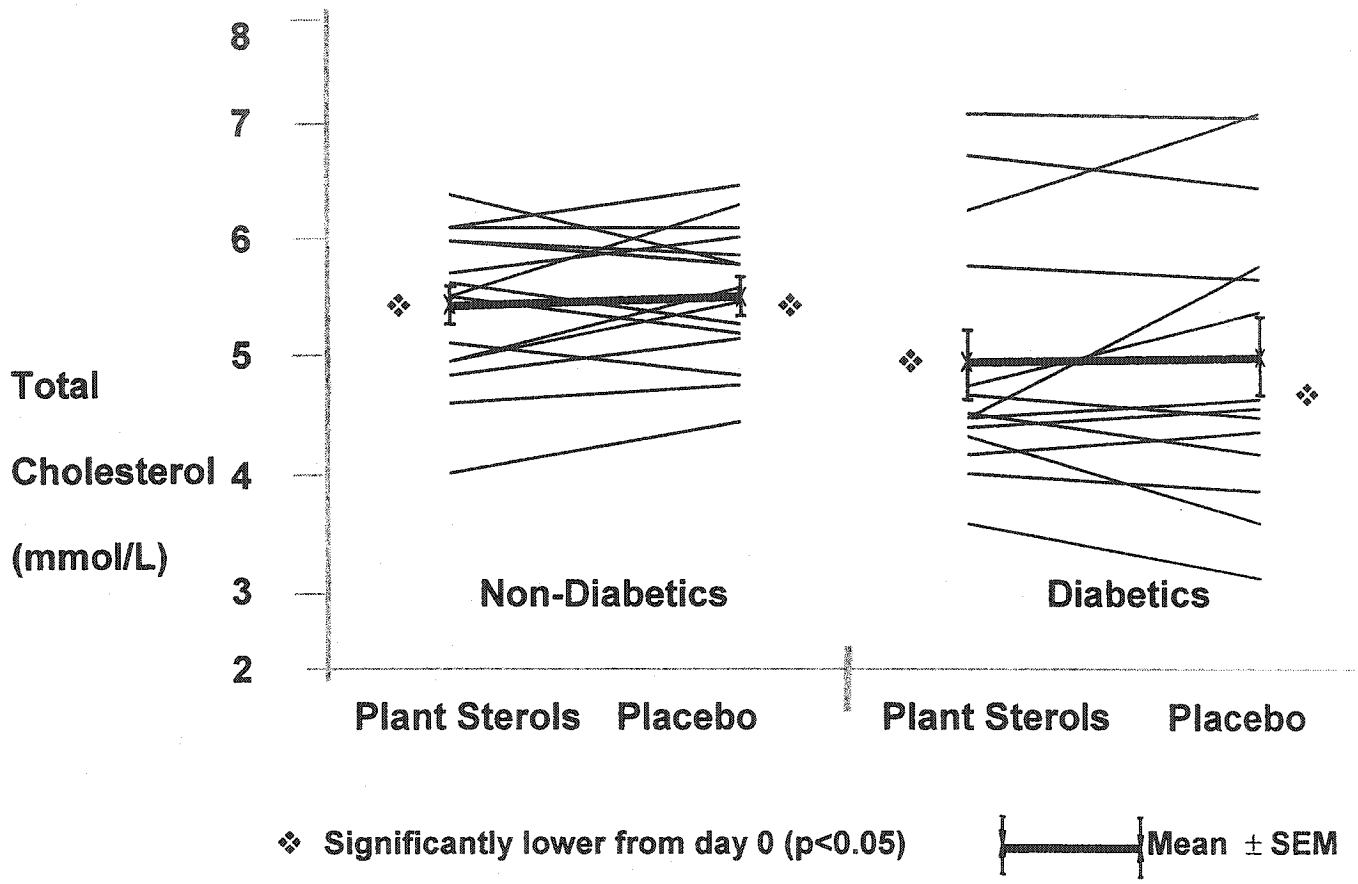

FIGURE 2 Effect of the plant sterol diet and placebo diet on end-point (day 21/22) total cholesterol concentrations for individual non-diabetic subjects (n = 15) and diabetic subjects (n = 14)

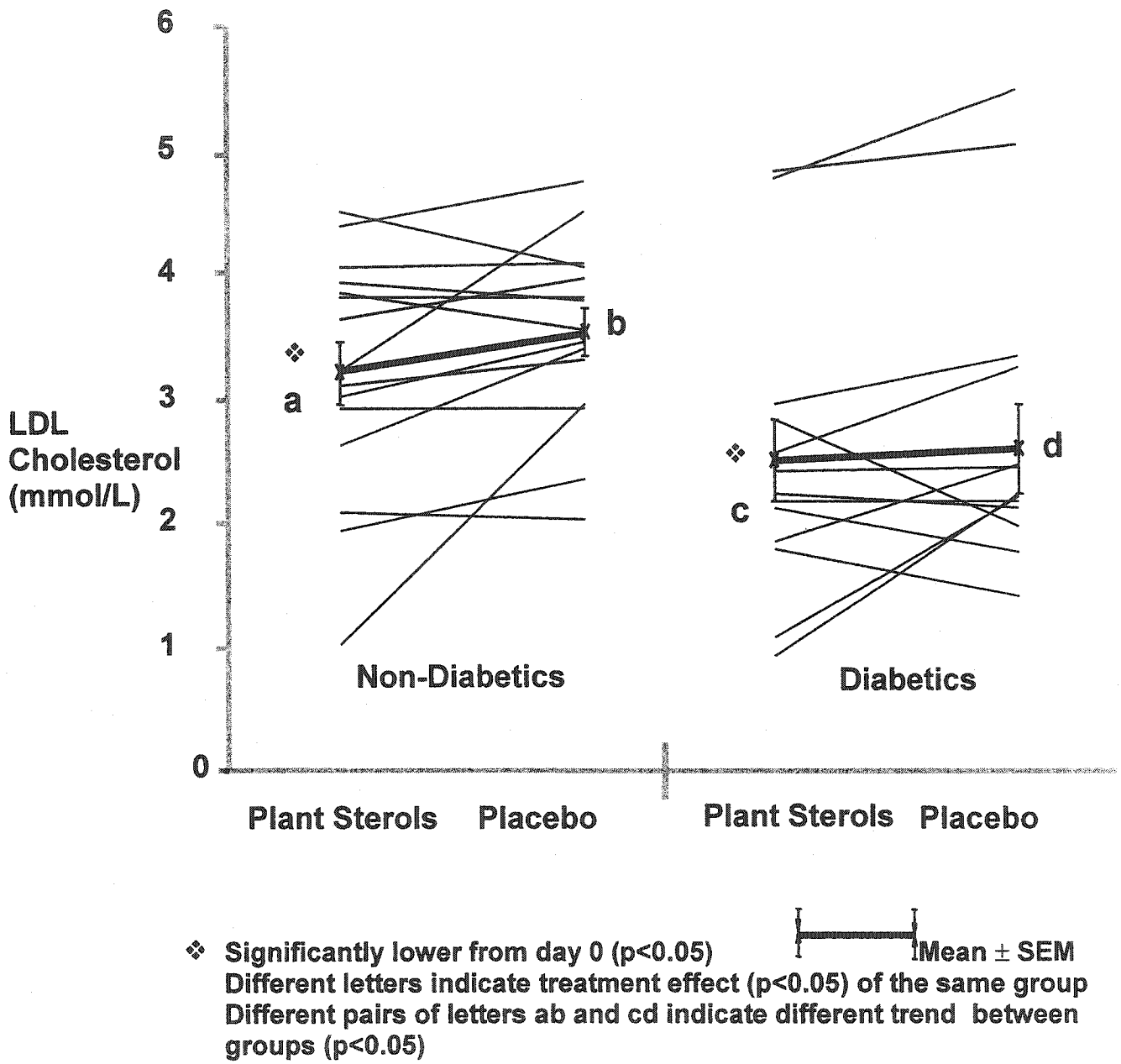

FIGURE 3 Effect of the plant sterol diet and placebo diet on end-point (day 21/22) LDL cholesterol concentrations for individual non-diabetic subjects (n = 15) and diabetic subjects (n = 13)

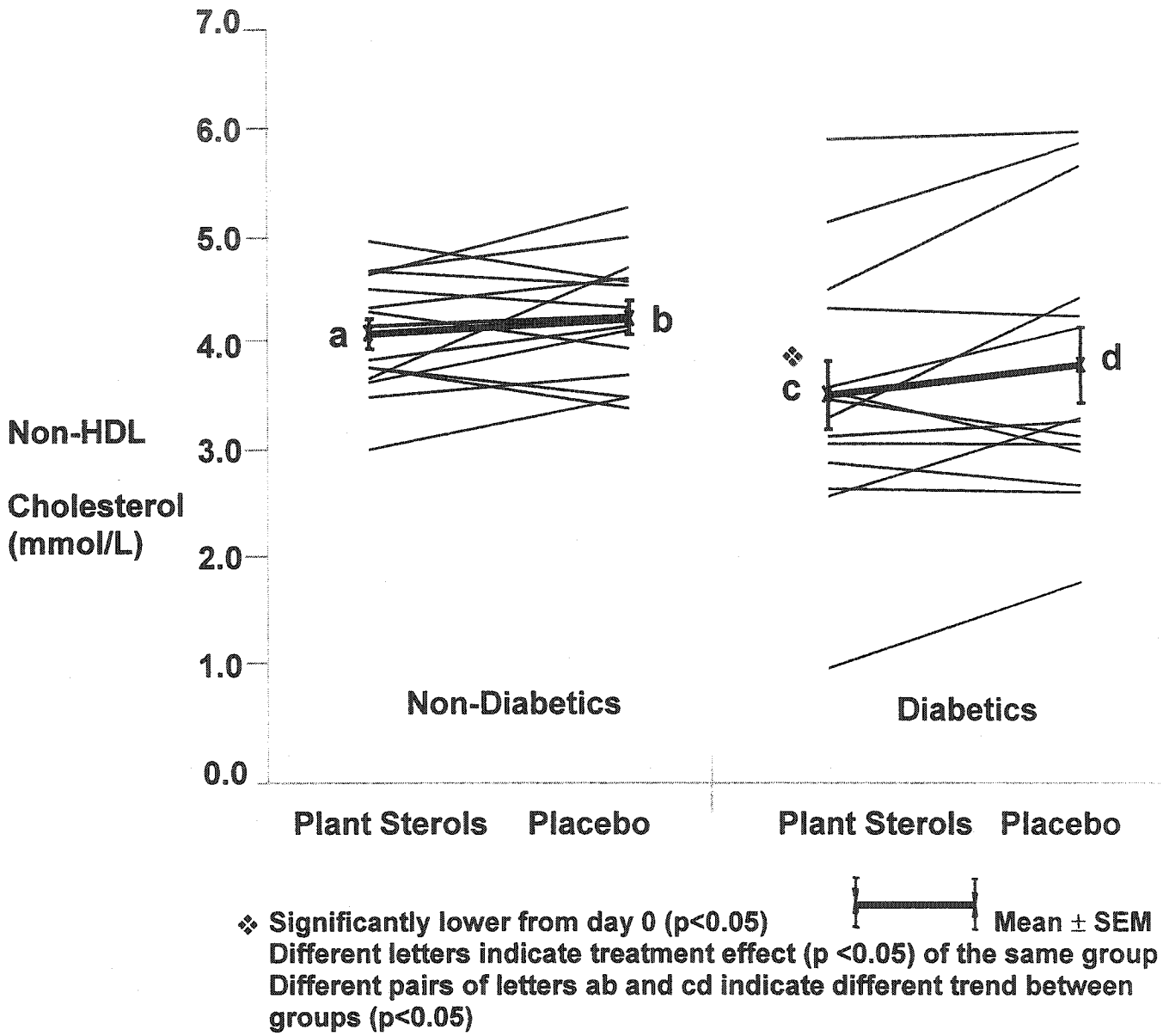

FIGURE 4 Effect of the plant sterol diet and placebo diet on end-point (day 21/22) non-HDL cholesterol concentrations for individual non-diabetic subjects (n = 15) and diabetic subjects (n = 14)

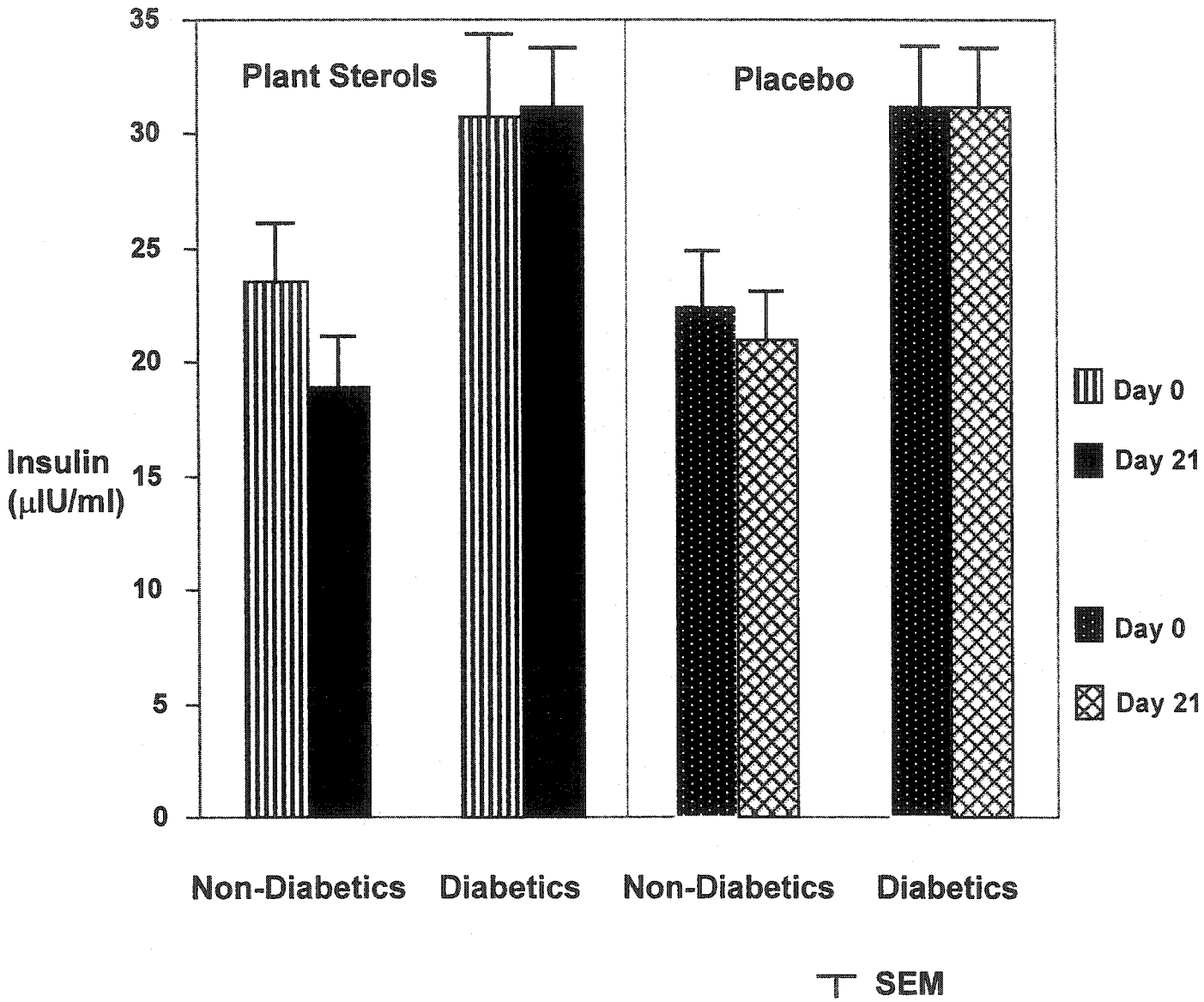

FIGURE 5 Effect of the plant sterol diet and placebo diet on baseline (day 0) and end-point (day 21) plasma insulin concentrations for non-diabetic subjects (n = 15) and diabetic subjects (n = 14)

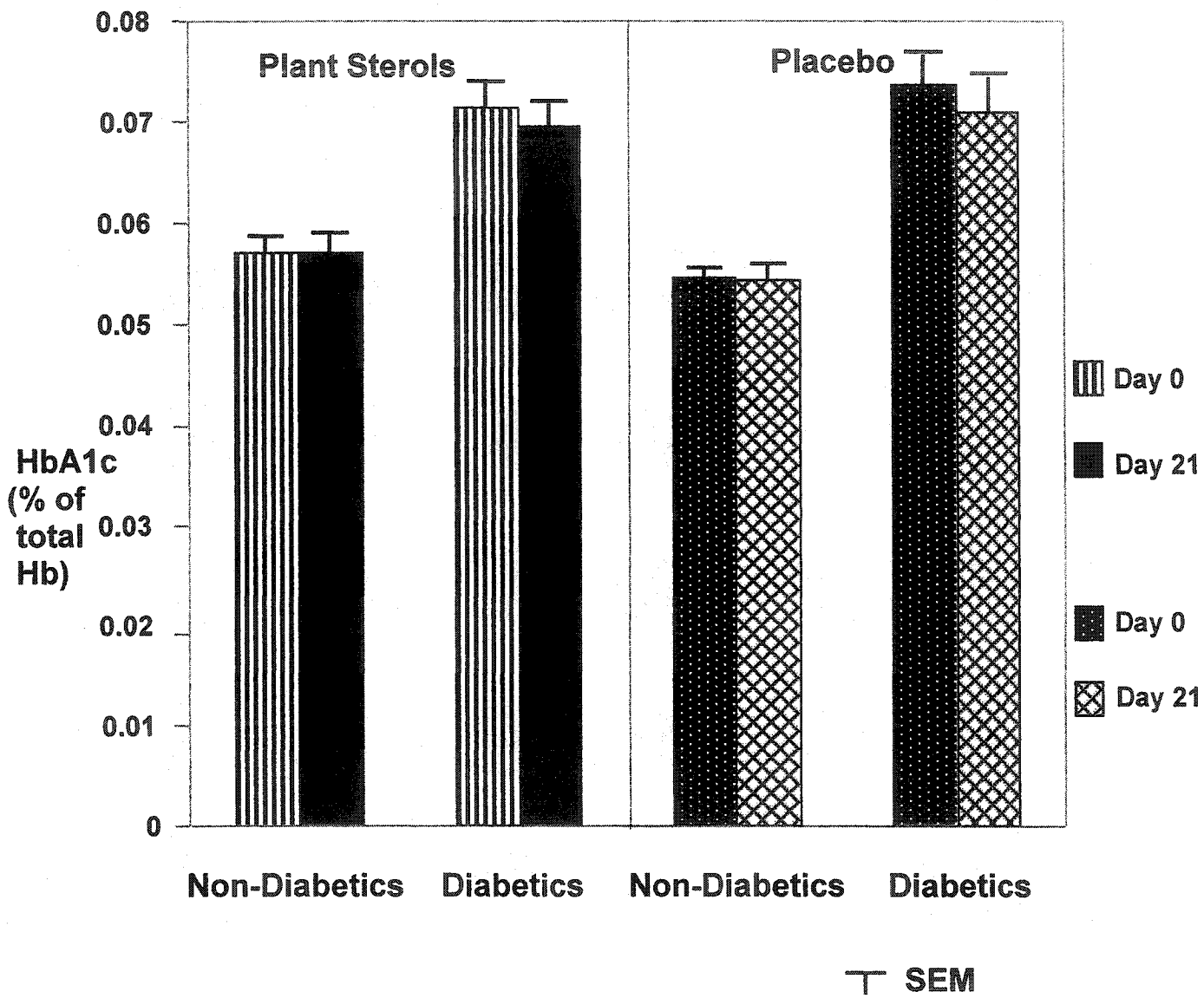

FIGURE 6 Effect of the plant sterol diet and placebo diet on baseline (day 0) and end-point (day 21) glycated hemoglobin concentrations for non-diabetic subjects (n = 10) and diabetic subjects (n = 10)

7. FINAL CONCLUSION

7.1 Summary of Results

Supplementing diets with PS for 21 days reduced plasma TC by 9.7% for non-diabetics and 13.7% for diabetics, LDL-C by 14.9% for non-diabetics and 29.9% for diabetics, non-HDL-C by 7.4% for non-diabetics and 18.6% for diabetics relative to baseline. Plasma LDL-C levels were lower ($p<0.05$) on PS vs. placebo phase, with the mean endpoint difference of 8.7% for non-diabetic subjects and 14.4% for diabetic subjects. Plasma non-HDL-C levels were lower ($p<0.05$) on PS vs. placebo phase, with the mean endpoint differences of 3.1% for non-diabetics and 10.7% for diabetic subjects. Dietary treatments did not affect HDL-C and TAG in plasma. There were no significant changes in plasma plant sterol levels after consumption of PS. Generally, fatty acid composition of RBC and body weight remained unchanged after consuming both PS and placebo diets indirectly indicated that all subjects had good compliance with both diets. In conclusion, consumption of a controlled diet supplemented with PS improved the overall cardiovascular risk profile of hypercholesterolemic individuals with and without type 2 diabetes.

7.2 Future Research

Demonstration for the first time that consumption of PS with or without a low saturated fat and low-cholesterol diet, has more improvement on the CVD risk profile in a population of moderately hypercholesterolemic type 2 diabetic individuals. This warrants further research on the possible role of PS in defining dietary strategies for best normalizing risk of CVD and associated complications in type 2 diabetics. Additional investigations with longer feeding trials, supplementing not only with PS but also with other proven hypocholesterolemic and/or hypoglycemic agents in separated phases, such as glucomanan and psyllium, should be considered. Excess body fat and sedentary lifestyle are the major notable modifiable risk factor of type 2 diabetes. Therefore, weight loss and exercise regimes can be potential adjunct to the dietary management of hyperlipidemia in type 2 diabetes.

Wagner et al. (2003) indicated that non-HDL-C and apolipoprotein B (apoB) seem to be equally useful in the detection of high-risk phenotypes in hypertriacylglycerolemic type 2 diabetic patients. Future studies with type 2 diabetic subjects should include apoB measurements along with plasma lipid profiles and non-HDL-C.

In order to monitor the effect of PS on glycemic control, fasting blood glucose and blood glucose 2 hours post meals should each be monitored at least once a week along with the weekly testing of plasma insulin, fructosamine and/or glycated hemoglobin. According to current dietary recommendations for type 2 diabetes, fruit juices from the 3-day cycle menu should be taken out for a better glycemic control.

Although the safety of PS has already been evaluated in previous studies with short feeding trials (Raeini-Sarjaz et al., 2002 and Christiansen et al., 2001) the variations in the levels of fat-soluble vitamins in plasma and in the stores should be measured if a longer feeding trial with PS is being conducted, i.e. a feeding trial of 3 months or more. This will determine whether a longer period of PS supplementation time will cause sub-clinical fat-soluble vitamin deficiencies or not.

As mentioned above, examining the separated and combined effects of supplementing PS, glucomannan, psyllium, weight loss and exercise regime along with precisely controlled diets, may provide more additive improvement in CVD risk in type 2 diabetic individuals because previous studies have examined each component and indicated benefits of each (Simonen, Gylling and Miettinen, 2002; Sierra et al., 2002; Krook et al., 2003; Vuksan et al., 1999)

7.3 Significance

This study for the first time demonstrates that consumption of PS is more efficacious in lowering LDL-C and non-HDL-C in type 2 diabetics compared to non-diabetic individuals. PS therefore serves as a potential adjunct to dietary management of hypercholesterolemia in type 2 diabetics. As the risk of developing CVD is two- to seven-fold higher in type 2 diabetics compared to non-diabetic individuals, this study demonstrates that PS consumption improves the excessive cardiovascular risk in this population. Therefore, incorporation of PS into the low saturated fat and low cholesterol diet of individuals at increased CVD mortality risk could have a positive impact on reducing the mortality rate of type 2 diabetes.

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APPENDIX I

SUBJECT CONSENT FORM OF RESEARCH PROTOCOL

The Effect of Phytosterols on Blood Glucose Level, Blood Lipid Profiles and Cholesterol Absorption, Synthesis and Turnover of Hypercholesterolemic Type II Diabetes Patient

Patient Name: _____ **Protocol Number:**_____

The Effect of Phytosterols on Blood Glucose Level, Blood Lipid Profiles and Cholesterol Absorption, Synthesis and Turnover of Hypercholesterolemic Type II Diabetes Patient

School of Dietetics and Human Nutrition, Macdonald Campus, McGill University.
Principal investigator: Dr. Peter Jones
Research Coordinators: Melanie Journoud and Vivian Wai-Yan Lau
Phone: (514) 398-7527
Contact Physician: Dr. William Parsons
Phone: (514) 694-4689

I, _____, the undersigned, hereby consent to participate as a subject in the above-named research project conducted by McGill University. The nature of the procedure or treatment, its risks and/ or benefits, and possible alternatives, follow:

I. NATURE AND DURATION OF PROCEDURE:

The aim of the study is to compare the cholesterol absorption, synthesis and turnover in hypercholesterolemic individuals with and without type II Diabetes consuming controlled solid foods diets for 3 weeks. Also, the relative impact of feeding phytosterols on blood lipid profiles, blood glucose levels, as well as apolipoprotein B and E, vitamin E, vitamin A and carotenoids, will be evaluated.

A fasting blood sample of 10 ml (two teaspoons) and a urine sample will be taken for the laboratory to confirm the absence of health abnormalities and to measure your fasting circulating lipid profiles and glucose. You will be permitted to take stable doses 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.

You will also need to ensure that you **REFRAIN** from (1) consuming any lipid lowering drugs, including fish oils or probucol within the last 6 months, (2) using insulin, systemic antibodies, corticosteroids, androgens, or phenytoin, (3) having myocardial infarction, coronary artery bypass, or other major surgical procedures within the last 6 months, (4) having recent onset of angina, congestive heart failure, inflammatory bowel disease, pancreatitis, or hypothyroidism, (5) having onset of significant gastrointestinal, renal, pulmonary, hepatic or biliary disease or cancer within the past 3 months and (6) using fiber or stimulant laxatives (greater than 2 doses/wk) or having a history of eating disorder or binging, or exercises greater than 15 miles/wk or 3000kcal/wk.

When you start the clinical trial, you will consume test diets provided by the Metabolic Kitchen at the Mary Emily Clinical Nutrition Research Unit for two (2) periods of 21 days. A period of four (4) weeks will separate each diet phase, where you will resume consumption of your typical intake. Each test diet will contain nutritionally adequate foods and be provided to you as three (3) meals per day. One of the diets will combine phytosterols, olive, flaxseed, canola, and safflower oil as a source of fat. This diet will contain 1.8 g/ day of plant sterol stanols, a material resembling cholesterol obtained from plant sources. These plant sterol stanols are tasteless and odorless.

During each 21-day dietary treatment phase, you will be required to consume your breakfast every weekday morning at the Research Unit. Lunch and supper can be packed and consumed away from the Research Unit. However, during each 21-day dietary treatment phase, you will be required to consume **ONLY** the food prepared at the Research Unit.

At the beginning (Day 1) and end (Day 22) of each dietary treatment phase, you will be examined by a physician to ensure that you are in good health. A portion of the blood sample taken across the study will be used to again confirm the absence of health abnormalities. At day 1, 8 and 15, you will be asked to provide 20 mL of blood for cholesterol level and blood analysis. In addition, at day 18, you will be injected with 15 mg of D7-cholesterol and you are required to provide 20 mL of blood at hours 0, 6 and 12 on day 18. In addition, you are required to provide fasting blood samples on days 19, 20, 21 and 22 (i.e. the morning after the final day of dietary phase) before breakfast to monitor the cholesterol enrichment or decay levels. At day 21, you are required to take approximately 25 ml of deuterium oxide orally before breakfast.

The total blood volume required for this trial will be 210 ml.

II. POTENTIAL RISKS AND/OR BENEFITS:

The dietary fats used in the preparation of the diets have all been approved for human consumption. The micronized plant sterol stanols added to the diet at the proposed has been shown to have no negative side effects on health in previous animal and human experiments. There is a slight chance you may experience mild stomach upset at the beginning of each dietary phase as your body adjusts to the new fat source. In case you feel any discomfort during the experimental trial a physician, Dr. Parsons, will be available to contact at any time. Dr. Parsons can be reached at (514) 694-4869.

The substance of the project and the procedures 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 procedures 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. Confidentiality of records concerning my involvement in this project will be maintained in an appropriate manner.

I, _____, have read the above description with one of the investigators, _____. I fully understand the procedures, advantages and disadvantages of the study, which have been explained to me. I understand that, in compensation for the inconvenience of the study schedule, I will receive $500 in two (2) installments of $250. I will also given access to my test results when they become available. If I

decide to withdraw before completion or should the study be terminated early, I will receive an appropriate pro-rated fraction of this amount.

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

Investigator	Signature of Subject
Witness	
Date	Time

FORMULAIRE DE CONSENTEMENT AU PROTOCOLE DE RECHERCHE

L'effet des phytostérols sur le taux de glucose sanguin, le profil lipidique et les taux d'absorption, de synthèse et de roulement du cholestérol chez des patients atteints d'hypercholestérolémie et de diabète de type II.

Nom du patient :_____ Numéro de protocole :_____

L'effet des phytostérols sur le taux de glucose sanguin, le profil lipidique et les taux d'absorption, de synthèse et de roulement du cholestérol chez des patients atteints d'hypercholestérolémie et de diabète de type II.

École de diététique et nutrition humaine, Campus Macdonald, Université McGill
Chercheur principal : Dr Peter Jones
Coordonnatrices de recherche : Mélanie Journoud, Vivian Wai-Yan Lau

Téléphone : (514) 398-7527
Médecin : Dr William Parsons
Téléphone : (514) 694-4689

Je, _____, sous-signé(e), consent à participer comme sujet au projet de recherche ci-haut mentionné, dirigé par l'Université McGill. La nature des procédures et des traitements, ses risques et/ ou bénéfices et alternatives possibles suivent :

I. NATURE ET DURÉE DES PROCÉDURES

Le but de cette étude est d'examiner l'effet d'une diète de 21 jours, contenant des phytostérols, sur les taux de glucose et de cholestérol sanguin, ainsi que sur l'absorption, la synthèse et le métabolisme du cholestérol chez des sujets qui ont un taux de cholestérol élevé et du diabète de type II, comparativement à des sujets qui ont seulement un taux de cholestérol élevé. L'étude permettra aussi de mesurer l'impact sur la vitamine E, la vitamine A et les caroténoïdes.

Un échantillon sanguin de 10 mL (deux cuillères à thé) et un échantillon d'urine seront pris pour vérifier, en laboratoire, l'absence de maladie et pour mesurer vos taux de glucose et de cholestérol.

Au cours de l'étude, vous aurez le droit de prendre des doses régulières des médicaments suivants : metformine, sulfonylurées, hormone thyroïde, anti-hypertensifs, et des hormones estrogènes pour les femmes ménopausées. Votre poids doit être stable avant de commencer l'étude.

Vous devrez vous **abstenir** de consommer: (1) des médicaments qui diminuent votre taux de cholestérol, incluant l'huile de poisson et le probucol, depuis les derniers 3 mois; (2) de l'insuline, des anticorps systémiques, des corticostéroïdes, des androgènes ou de la phénytoïne; (3) des fibres ou laxatifs stimulants (plus de 2 doses par semaine). Si vous êtes un(e) patient(e) diabétique, votre médicament et sa dose doivent être les mêmes depuis les derniers 3 mois.

Vous ne pouvez pas avoir subi ou avoir souffert (1) d'un infarctus; (2) de pontage coronarien, artériel ou autres chirurgies majeures durant les derniers 6 mois; (4) d'épisodes récents de crises d'angine, d'insuffisance cardiaque, d'inflammation du tube digestif, de pancréatite ou d'hypothyroïdie; (5) de maladies gastro-intestinales, rénales, pulmonaires, hépatiques ou biliaires, d'un cancer depuis les derniers 3 mois; (6) de troubles alimentaires tels que l'anorexie ou la boulimie; (7) d'efforts physiques supérieurs à 15 miles/ semaine ou équivalents à plus de 3000 kcal/ semaine.

Dès que l'étude aura débutée, vous devrez consommer les repas fournis par la clinique durant deux (2) périodes de 21 jours. Une période de 28 jours séparera chaque période, pendant lesquelles vous retournerez à votre diète habituelle. Chaque diète expérimentale sera composée d'aliments habituels qui vous seront fournis en trois (3) repas par jour. Une des diètes contiendra une combinaison d'huile d'olive, de phytostérols, d'huile de lin et d'huile de canola et d'huile de carthame comme sources principales de gras. Cette diète fournira en tout 1.8 grammes de phytostérols par jour. Les phytostérols sont l'équivalent du cholestérol humain chez les plantes. Ils sont sans saveur, ni odeur.

Durant chaque période de 21 jours, vous devrez prendre tous les repas qui vous seront fournis par la clinique. Le déjeuner devra être consommé tous les matins à la clinique, à l'exception des fins de semaine. Le reste des repas sera emballé pour consommer à l'endroit de votre choix. Durant ces 21 jours, **SEULS** les repas fournis par la clinique pourront être consommés.

Au début (jour 1) et à la fin (jour 22) de chaque période de traitement, vous devrez passer un examen médical pour confirmer votre état de santé. Aux jours 1, 8 et 15, vous devrez fournir 20 mL de sang pour des analyses de cholestérol et autres tests sanguins. De plus, au jour 18, vous devrez subir une injection de 15 mL (1 cuillère à soupe) de marqueur de cholestérol D7. Vous devrez ensuite fournir 20 mL de sang à l'heure 0, 6 et 12 ce même jour. Au jour 21, vous devrez prendre 25 mL par voie orale de l'oxyde de deutérium, un second marqueur de cholestérol. Vous devrez fournir des prises de sang de 20 mL aux jours 19, 20, 21, et 22.

Le total de sang prélevé pour l'étude sera de 210 mL.

II. RISQUES ET/OU BÉNÉFICES POTENTIELS

Les huiles utilisées dans la préparation des diètes sont toutes approuvées pour consommation par les humains. L'ajout de phytostérols à la diète dans les concentrations ci-haut mentionnées n'ont aucun effet négatif sur la santé des humains ni des animaux. Il y a une faible chance que vous ayez quelques dérangements intestinaux au début de chaque phase avant que votre corps ne s'habitue à la nouvelle source de gras dans votre diète. En cas de malaises durant l'étude, un médecin, Dr Parsons, sera disponible en tout temps. Dr Parsons peut être rejoint au : (514) 694-4869.

Le but du projet de recherche et les procédures qui y sont associés m'ont été expliqués et toutes les procédures expérimentales décrites. J'ai eu l'opportunité de poser des questions concernant tous les aspects du projet et des procédures. Je suis conscient(e) que je peux refuser de participer et que je peux retirer mon consentement en tout temps. J'affirme qu'aucune garantie ne m'a été donnée quant aux résultats à venir. La confidentialité des dossiers concernant mon implication dans ce projet sera maintenue de façon appropriée.

J'ai, _____, pris connaissance des descriptions ci-haut avec un des chercheurs, _____. Je comprends entièrement les procédures, avantages et désavantages entourant l'étude, et ceux-ci m'ont été expliqués. Je reconnais qu'en compensation des inconvénients encourus en participant à cette étude, je recevrai 500 $ en deux (2) versements de 250 $. J'aurai accès à mes résultats de tests lorsqu'ils seront disponibles. Si je décide de démissionner avant la fin de l'étude, ou si celle-ci devait se terminer plus tôt, je recevrai un paiement au prorata de la fraction complétée.

Je reconnais avoir reçu une copie de ce formulaire de consentement et tous les feuillets joints et consent à être contacté par un membre du comité d'éthique sur la recherche.

_____	_____
Chercheur	Sujet
_____	
Témoin	
_____	_____
Date	Heure