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ORIGINAL CONTRIBUTION

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A phytosterol-enriched spread improves the lipid profile of subjects with type 2 diabetes mellitus

A randomized controlled trial under free-living conditions

Summary *Background* Phytosterol-enriched margarines are known to significantly lower total and LDL cholesterol, but little is known about the effect of such margarines in subjects with type 2 diabetes. *Aim of the study* Investigation of the effect of a phytosterol-enriched spread in subjects with type 2 diabetes mellitus on serum lipids, Hb_{A1c}, and blood glucose under free-living conditions. *Methods* Randomized, placebo-controlled, double-blind clinical trial in two parallel groups over 12 weeks; 85 type 2 diabetic patients with serum LDL cholesterol levels ≥ 3.60 mmol/l and without hypolipidemic medication were included in the study. Participants consumed 2 x 10 g of spread with or without 8 % phytosterol-esters daily. Fasting blood samples were analyzed at 0, 4, 8, and 12 weeks. *Results* After 4 weeks, total and LDL cholesterol were significantly reduced in the phytosterol group by 5.2 % and 6.8 %, respectively, compared to baseline ($p < 0.05$). After 8 and 12 weeks, these reductions became smaller and were not significant any more compared to baseline or between the groups, but a repeated measurement analysis demonstrated a significant difference for both variables between the two groups (each $p < 0.05$). HDL cholesterol was significantly increased in the phytosterol group compared to the placebo group after 8 and 12 weeks, but there was no overall difference in the repeated measurement analysis between the two groups. In the phytosterol group, there was a small reduction in Hb_{A1c} compared to the control group which was only significant after 4 weeks. *Conclusions* This clinical study shows that a phytosterol-enriched spread is effective in lowering total and LDL cholesterol in subjects with type 2 diabetes but also illustrates the difficult maintenance under free-living conditions over time. Although this effect is modest, it may contribute to decreasing the elevated risk of cardiovascular disease in type 2 diabetes.

Key words type 2 diabetes mellitus – phytosterol – LDL cholesterol – hypercholesterolemia – phytosterol-enriched spread

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Introduction

Elevated serum levels of total and LDL cholesterol are well-known potent risk factors for cardiovascular disease in the general population [1] as well as in subjects with diabetes mellitus [2, 3]. The absolute risk of developing cardiovascular disease is two- to seven-fold higher in diabetic compared to non-diabetic individuals [2–4]. For this reason, recent recommendations propose to strive for similar blood lipids in diabetic subjects as those recommended for the secondary prevention in non-diabetic persons after a myocardial infarction. Recent intervention trials with statins in type 2 diabetic patients were able to demonstrate a significant decrease in the risk of cardiovascular complications [5–8]. In the

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subgroup analysis of the 4S study the risk reduction in the diabetic group was even greater than in the non-diabetic group [6].

As early as the 1950s, it was recognized that phytosterols have cholesterol lowering properties [9, 10]. Serum cholesterol concentrations are probably lowered by a reduction in the absorption of cholesterol in the gut. Due to their structural similarities phytosterols compete with cholesterol for the inclusion into mixed micelles. β-Sitosterol, the main member of the phytosterol family, was used for many years in the treatment of hypercholesterolemia [11]. As high doses of the crystalline form of phytosterol were required to effectively decrease blood cholesterol and due to the approval of the highly effective statins, β-sitosterol was rarely used as a cholesterol-lowering medication. It was only recently that the cholesterol-lowering potential of phytosterol was rediscovered when spreads were developed which contained phytosterol- or phytostanolesters, the hydrogenated form of sterols. Spread is a good vehicle to solubilize phytosterol- and phytostanolesters and even the small amounts added were able to significantly lower serum cholesterol [12–25]. Little is currently known about the potential benefit of phytosterol-enriched spread in patients with type 2 diabetes [12, 14], and nothing is known about the effect of this spread under free-living conditions. In addition, it is completely unclear whether phytosterol-enriched spreads have any effect on the metabolic control of subjects with type 2 diabetes. Therefore, the objectives of this study were a) to examine the potential of a phytosterol-enriched spread to lower total and LDL cholesterol in subjects with type 2 diabetes with moderate hypercholesterolemia, b) to study the efficacy of the spread under real-life conditions and c) to assess whether the phytosterol-enriched spread has an effect on parameters of glycemic control.

Subjects and methods

Subjects

The participants were recruited by advertisements in local newspapers, at the out-patient unit of the German Diabetes Research Institute in Düsseldorf, and at the practices of diabetologists. One hundred and eighty four subjects were included in the screening procedure. A total of 85 subjects met the eligibility criteria and were enrolled. The primary selection criteria were type 2 diabetes for at least one year with a stable medication, serum LDL cholesterol concentration ≥ 3.60 mmol/l, no treatment with hypolipidemic agents, and not being on a hypocaloric diet. Fifty participants habitually used margarine, 26 used butter, and 9 subjects did not use spread before entering the study. However, the latter ate cheese, liverwurst, or other fatty spreads and were willing to use the margarine for the 3-month period. The clinical characteristics of the participants who completed the intervention are presented in Table 1.

Safety parameters such as hemoglobin, creatinine, potassium, aspartate aminotransferase, and alanine aminotransferase were within normal range at baseline.

All participants gave their written consent prior to the beginning of the study. The study protocol was approved by the Ethical Committee of the Heinrich-Heine University Düsseldorf.

Experimental design

This was a randomized, double-blind, placebo-controlled study in two parallel groups over a period of 12 weeks. The volunteers received coded tubs containing 10 g of either a low-fat spread (becel®) or a phytosterol-enriched low-fat spread (becel® pro.active). The spreads were produced and provided by Unilever (Hamburg, Germany). The composition of the spreads is shown in Table 2. Participants were asked to consume 2 tubs daily

Table 1 Clinical characteristics of the 81 type 2 diabetic patients at baseline

Variable	Treatment group	
	Placebo	Phytosterol-enriched spread
N	40	41
Age (years)	62 ± 6	60 ± 8
Gender (female/male)	23/17	22/19
Weight (kg)	84.1 ± 12.0	81.9 ± 13.8
BMI (kg/m^2)	29.9 ± 5.3	28.3 ± 4.7
Antidiabetic medication	30 (75%)	34 (82.9%)
sulphonylureas, metformin, acarbose, alone or in combination with insulin	26 (65%)	21 (61%)
insulin alone	4 (10%)	9 (21.6%)

Continuous variables are presented as means ± SD

Table 2 Composition of the low-fat spread and phytosterol-enriched low-fat spread in the daily portion of 20 g

	Placebo = becel®	Phytosterol-enriched spread = becel pro.active®
Energy	296 kJ/72 kcal	296 kJ/72 kcal
Protein (g)	0	0
Carbohydrate (g)	0	0
Fat (g)	8	8
Saturated FA (g)	1.8	1.8
MUFA (g)	2.2	2.2
PUFA (g)	4	4
Phytosterol (g)	~ 0.08	≅ 1.6
Vitamin E (mg)	12.4	12.4
Vitamin A (µg)	180	180
Vitamin D (µg)	0.46	0.46

(2 x 10 g) which was equivalent in the phytosterol-enriched version to 1.6 g phytosterols (8 %) as a bread spread for breakfast and for dinner replacing their usual spread or butter while maintaining their eating habits. Patients did not receive additional dietary counseling. Dietary intake was assessed using a structured dietary intake questionnaire [26]. In this questionnaire food is divided into three categories such as green for food that should be preferred, red for food that should be rather avoided, and red-green food that should be consumed seldom and with care. Possible changes in dietary intake were registered by comparing the difference of the green, red, and red-green food at the beginning and at the end of the study period in each group. The participants were asked to maintain their lifestyle and diabetes diet and keep their body weight stable. Blood samples were taken at weeks 0, 4, 8, and 12 after an overnight fast and immediately analyzed. The patients received 60 tubs of prepared spread at week 0, 4, and 8 for the coming four weeks. Patient compliance was monitored by registration of the number of unused tubs, which the patients were asked to bring in, adverse effects were documented at each visit as well as changes in body weight, lifestyle, medication, and intercurrent illnesses.

Methods

The laboratory analysis of the blood samples was conducted at the Department of Clinical Biochemistry of the German Diabetes Research Institute. Serum total, HDL cholesterol and triglycerides were measured with commercially available kits (Roche Diagnostics, Mannheim, Germany). The formula of Friedewald et al. [27] was used to calculate LDL cholesterol. Hb_{A1c} was measured chromatographically on a Diamat™ (Bio-Rad, Munich, Germany). Fructosamine was analyzed using a commercially available kit (Roche Diagnostics, Mannheim, Germany). Whole blood glucose concentrations were measured with the hexokinase method.

Statistical analysis

Continuous variables were described by mean ± standard deviation. In the graphical presentation of the continuous variables means and 95 % confidence intervals were used. Differences in changes between both groups at fixed times were compared by unpaired 2-sided t-tests and changes within groups compared to baseline by paired 2-sided t-tests. In all statistical tests, the level of significance was defined as $p < 0.05$.

To assess the overall effect of the treatment, repeated measurement analysis of variance was performed using the original values of the variables at all time points. To adjust for the initial values, the baseline variable was included as covariate in a further model. The p-values of the multivariate tests of the interaction between time and treatment group were presented. The data analysis was performed using the statistical software package SAS version 6.12 TS 020.

Results

Eighty one subjects, 40 in the control group and 41 in the group taking phytosterol-enriched spread, completed the study. The two groups were comparable with regard to age, gender distribution, weight, and BMI. At study entry 75 % (n = 30) of the participants in the placebo group and 82.9 % (n = 34) in the phytosterol group received antidiabetic medication (Table 1). Out of the original 85 participants two dropped out due to poor compliance and another two due to dislike of the taste of the spread. The spreads were overall well tolerated. In the placebo group, one person mentioned occasional heartburn and another reported about flatulence in the first week. In one case, nausea was reported in the first days in the group receiving the phytosterol-enriched margarine. According to the given information obtained at the patient visits, 2 tubs of the spread were used daily in both groups (data not shown). Body weight of the patients did not change during the intervention. In the phytosterol group, the mean BMI was 28.3 ± 4.7 kg/m^2 at the beginning and 28.3 ± 4.6 kg/m^2 at the end of the study and in the control group 29.9 ± 5.3 kg/m^2 and 29.9 ± 5.1 kg/m^2, respectively. Nutrition assessment revealed a minor change in food choice for both groups with no statistical significance between the groups (data not shown).

Effect on plasma lipids

At baseline total, LDL, HDL cholesterol, and fasting triglyceride levels were comparable between the two groups (Table 3). At week 4, compared to baseline a reduction of total and LDL cholesterol by 0.34 ± 0.64 mmol/l and 0.30 ± 0.62 mmol/l, respectively, was observed in the phytosterol group corresponding to relative reductions of 5.2 % and 6.8 %, respectively (each $p < 0.05$). In the placebo group, total and LDL cholesterol were slightly elevated compared to baseline values by 0.07 ± 0.54 mmol/l and 0.06 ± 0.53 mmol/l, respectively, corresponding to relative elevations of 1.1 % and 1.5 %, respectively. At week 8 and 12, the reduction in the phytosterol-treated group became smaller and was no longer significantly different from baseline values or from the placebo group (Fig. 1). In the repeated measurement analysis of variance, the change of total and LDL cholesterol was significantly different between the groups overall ($p = 0.003$ and $p = 0.027$, respectively), even after adjustment for baseline values ($p = 0.002$ and $p = 0.047$, respectively).

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Table 3 Serum lipids during the 12-week consumption of a phytosterol-enriched low-fat spread vs. a normal low-fat spread in subjects with type 2 diabetes

	Total cholesterol (mmol/l)	LDL cholesterol (mmol/l)	HDL cholesterol (mmol/l)	LDL/HDL ratio	Triglycerides (mmol/l)
Placebo					
Baseline	6.42 ± 0.81	4.11 ± 0.73	1.37 ± 0.37	3.18 ± 0.92	2.26 ± 1.36
4 weeks	6.49 ± 0.71	4.16 ± 0.64	1.37 ± 0.40	3.23 ± 0.89	2.12 ± 0.99
8 weeks	6.25 ± 0.80[a]	4.03 ± 0.68	1.33 ± 0.36	3.22 ± 0.90	1.96 ± 0.88
12 weeks	6.36 ± 0.67	4.01 ± 0.63	1.40 ± 0.42	3.08 ± 0.94	2.24 ± 1.39
Phytosterol-enriched spread					
Baseline	6.46 ± 0.83	4.33 ± 0.65	1.25 ± 0.37	3.74 ± 1.19	1.99 ± 1.01
4 weeks	6.13 ± 0.98*, [a]	4.03 ± 0.78 *, [a]	1.29 ± 0.36	3.33 ± 1.04*, [a]	1.77 ± 1.03[a]
8 weeks	6.19 ± 1.12[a]	4.06 ± 0.94	1.32 ± 0.40*, [a]	3.31 ± 1.22*, [a]	1.78 ± 0.99[a]
12 weeks	6.28 ± 1.00	4.13 ± 0.82	1.31 ± 0.38[a]	3.35 ± 1.02[a]	1.84 ± 1.04

Data are means ± SD.
* change from baseline significantly different between placebo and phytosterol group ($p < 0.05$)
[a] significantly different from baseline ($p < 0.05$)

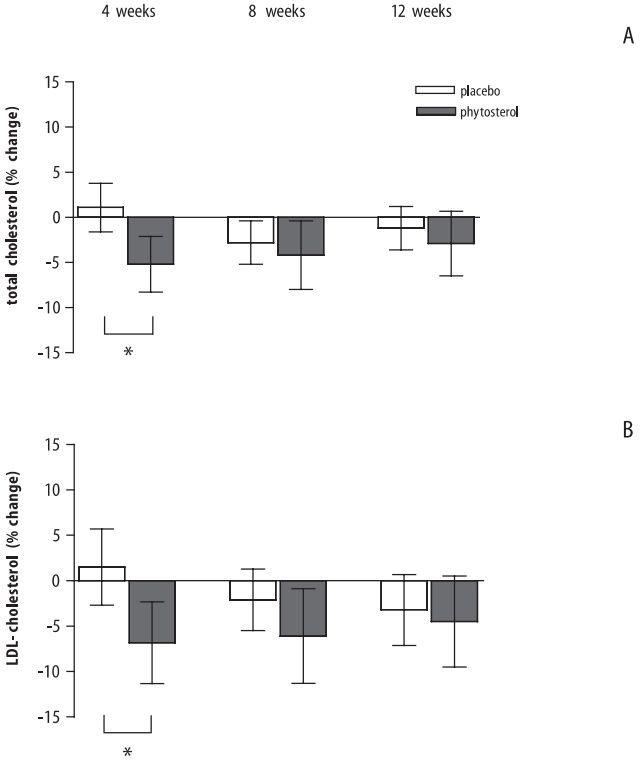

Fig. 1 Effect of a phytosterol-enriched spread versus a control spread on total (**A**) and LDL cholesterol (**B**) in type 2 diabetic subjects during a 3-month administration. Data are means of the relative change versus baseline and 95 % confidence intervals. * $p < 0.05$ phytosterol group versus placebo group as well as phytosterol group 4 weeks vs baseline

There was a significant elevation of HDL cholesterol in the phytosterol group versus baseline after 8 and 12 weeks. The HDL cholesterol change from baseline was significantly different between the groups after 8 weeks. However, the repeated measurement analysis did not show a significant difference between the two groups ($p = 0.057$, after adjustment for initial values $p = 0.093$). In contrast, the change in the LDL/HDL ratio versus baseline was significantly different between the two groups overall (repeated measurement analysis: $p = 0.007$, after adjustment for initial values $p = 0.036$). The changes versus baseline in the LDL/HDL ratio at the various time points are shown in Table 3. Concerning fasting triglycerides, no significant change between groups was detected in the repeated measurement analysis ($p = 0.282$ and $p = 0.300$, respectively). However, fasting triglycerides in the phytosterol group were significantly lower after 4 and 8 weeks compared to baseline (Table 3).

Effect on glycemic control

The level of glycemic control was good to moderate at baseline with mean fasting blood glucose values of 8.6 ± 2.3 mmol/l and mean Hb_{A1c} of 7.6 ± 1 % in the placebo group and 8.3 ± 2.8 mmol/l and 7.6 ± 1.4 %, respectively, in the phytosterol group. Repeated measurement analysis of fasting blood glucose concentrations did not detect a significant difference between the groups over time ($p = 0.765$, after adjustment for initial values, $p = 0.619$). Likewise, repeated measurement analysis of Hb_{A1c} did not show a significant difference between the groups over time ($p = 0.163$, after adjustment for initial values $p = 0.160$). However, a small improvement in glycemic control was found after 4 and 8 weeks with reductions of Hb_{A1c} versus baseline in the phytosterol group by 0.28 ± 0.46 % and 0.30 ± 0.62 %, respectively. The difference in change between the two groups was statistically significant only after 4 weeks ($p < 0.05$). Fructosamine concentrations were also slightly reduced compared to baseline but did not reveal

significant differences compared to baseline or between groups (Fig. 2).

Discussion

In this randomized, double-blind, placebo-controlled parallel design study over a period of 12 weeks, the phytosterol-enriched spread was effective and could demonstrate for the first time an improvement of total and LDL cholesterol in a large group of patients with type 2 diabetes under free-living conditions. The results of this study are in good agreement with a number of studies of various designs using phytosterol- or phytostanol-esters which described decreases in total and LDL cholesterol in the range of 6–10.2 % and 5.6–24.4 %, respectively [12–17, 19–25]. In our study in subjects with type 2 diabetes, total cholesterol decreased by 5.2 % and LDL cholesterol by 6.8 % after 4 weeks versus baseline, which is at the lower limit of this range. Although the repeated measurement analysis showed significant differences between the two groups, the reduction in total and LDL cholesterol, respectively, was significant by different from the control group only at 4 weeks, but after 8 and 12 weeks of administration of the phytosterol-enriched spread the difference between both groups became smaller indicating that the initial effect was not maintained.

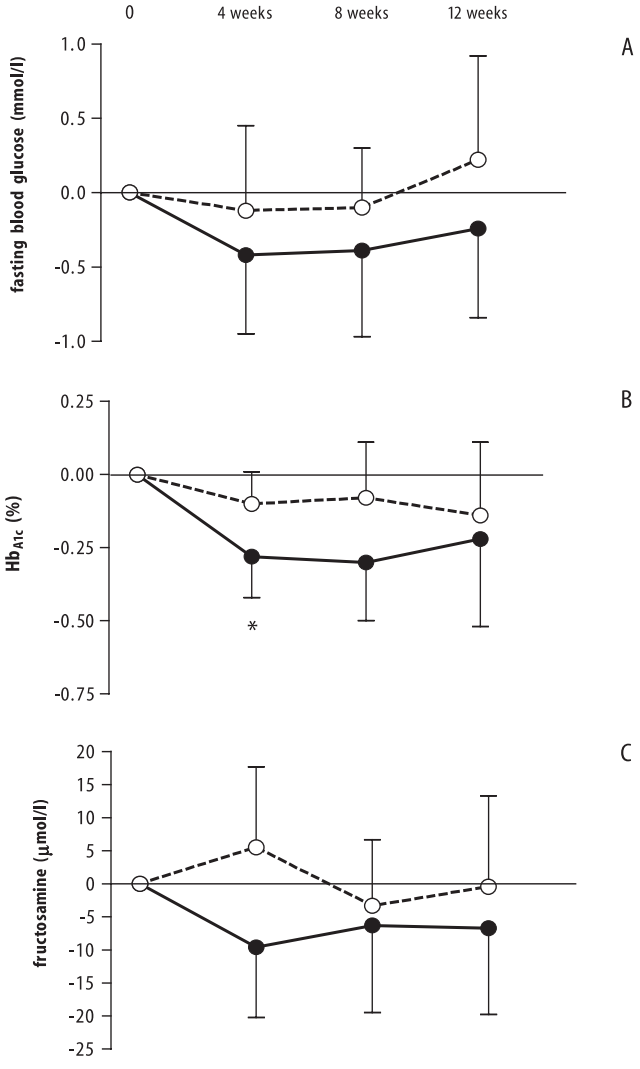

Fig. 2 Fasting blood glucose (**A**), glycated hemoglobin (**B**), and fructosamine (**C**) concentrations during a 3-month administration of 20 g phytosterol-enriched spread daily vs. a low-fat spread. Data represent means of change and 95 % confidence intervals, * $p < 0.05$ phytosterol group (●—●) versus placebo group (○---○)

Several factors may explain this relatively modest and transient effect compared to previous studies in non-diabetic subjects. The calculated phytosterol intake of 1.6 g/d in this study was relatively low compared to most above mentioned studies. A previous study could show a dose-dependency of the cholesterol-lowering effect of phytosterol-esters [21]. Therefore, it appears possible that a higher intake may also result in a greater reduction in total and LDL cholesterol, particularly in this type of patients. In most previous studies [17, 19–21, 24] participants were either given prepared defined meals under supervision or received intensive dietary counseling to keep a fat-reduced diet. In our study, the patients with type 2 diabetes were asked to maintain their lifestyle and habitual diet and only replace their spread. The emphasis lay on the efficacy of the spread under free-living conditions portraying realistic everyday life conditions.

Another explanation could be the complexity of the disturbances in lipoprotein metabolism in type 2 diabetes which is distinctly different from individuals without diabetes [28]. However, this is only speculative and should be tested in an appropriate clinical study. Another explanation for the wearing off of this cholesterol-lowering effect is a decrease in compliance for spread intake. The assessment of compliance is generally very critical. Patients were asked to return the unused tubs at each visit and this number was registered. However, this technique only allows a statement about the number of returned tubs but it does not provide information as to whether the margarine was really consumed completely. Although the patients were asked at each visit about their consumption of the spreads, the answers may be unreliable. To date, there is no evidence from other studies that the cholesterol lowering potential wears off, if a phytosterol-enriched spread is used for an extended period of time. However, these studies were short-term and were scheduled for an intake of the phytosterol-enriched spread over only a few weeks.

Although the maximum decrease in LDL cholesterol by 6.8 % appears to be small, it should be kept in mind that in dietary intervention trials in free-living subjects

the cholesterol-lowering effect of usual dietary counseling was in the same range or even smaller [29]. However, there is some evidence that this limited reduction may be beneficial in terms of cardiovascular disease risk reduction [23]. Nevertheless the use of a phytosterol-enriched low-fat spread could be particularly attractive and cost-effective in diabetic subjects as the risk of developing cardiovascular complications is markedly elevated in this patient group [2–4].

Our data also suggest a minor positive effect on HDL cholesterol, as serum concentrations were significantly increased in the phytosterol group after 8 and 12 weeks. A similar finding has been reported in a previous study using phytostanol-ester-enriched spread in patients with type 2 diabetes [11] indicating that in this particular group a phytosterol-enriched spread not only lowers LDL cholesterol but may have additional favorable effects on other variables of the disturbed lipoprotein metabolism. An increase in HDL cholesterol is particularly desirable in subjects with type 2 diabetes as they frequently have low HDL cholesterol levels [2, 3, 28]. However, as the repeated measurement analysis showed no significant differences from the control group over time, this finding should not be overinterpreted and may be of limited clinical significance.

Another objective of this study was to examine possible effects of phytosterol-esters on glycemic control as nothing is known about this question, and glucose and lipoprotein metabolism are closely interrelated in type 2 diabetes [28]. The glycemic control improved slightly in the subjects using phytosterol-enriched spread even though the repeated measurement analysis did not reveal significant differences over time. Although the effect was rather modest, it is interesting and novel and may require additional studies for verification.

In conclusion, the present study demonstrates for the first time that a phytosterol-enriched spread is effective in lowering total and LDL cholesterol in a large group of patients with type 2 diabetes. However, the loss of effect in the course of the study clearly illustrates the difficulties to maintain this effect under free-living conditions over an extended period of time. Interestingly, there was preliminary evidence for a small improvement in triglycerides metabolism and glycemic control. Irrespective of the clinical significance of the latter finding, the use of a phytosterol-enriched spread may contribute to lower cholesterol levels and may, thereby, decrease the excessive risk of cardiovascular complications in patients with type 2 diabetes.

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