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ORIGINAL CONTRIBUTION

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Plant stanol esters in low-fat milk products lower serum total and LDL cholesterol

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Abstract *Background* Oil-based matrices enriched with plant stanol esters lower serum LDL cholesterol. The effects of low-fat milk products have been less thoroughly examined. *Aim of the study* To evaluate the effect of three less explored low-fat milk products enriched with plant stanol esters on serum lipid concentrations in subjects with mild or moderate hypercholesterolemia. *Methods* A meta-analysis of four unpublished sub-studies (yoghurt, yoghurt single-shot drink: Studies I and II, or milk). All the sub-studies were randomized, placebo-controlled, double-blind and had a parallel-group design. They were carried out in order to evaluate the effect of low-fat milk products enriched with plant stanol esters on serum lipid concentration. Each stanol-ester-enriched milk product provided 2 g of stanols per day, and in each study the intervention period was 5 weeks. A total of 199 hypercholesterolemic subjects completed the studies. *Results* The pooled treatment difference in total cholesterol was −3.8% (95% CI −6.0 to −1.7, $p < 0.001$) when stanol was compared to placebo. In LDL cholesterol, the pooled treatment difference was −4.9% (95% CI −7.8 to −1.8, $p = 0.002$). There were no significant differences between the groups in pooled HDL cholesterol or triacylglycerol concentrations. The results tended to be more pronounced when we were certain that the yoghurt single-shot drink was ingested with lunch, and when the baseline LDL-cholesterol concentration was ≥3.5 mmol/l. *Conclusions* These results imply that low-fat milk products enriched with plant stanol esters lower both total cholesterol and LDL cholesterol statistically significantly in subjects with mild or moderate hypercholesterolemia. The changes tended to relate to the baseline LDL-cholesterol concentration.

Key words phytosterols – plant stanols – hypercholesterolaemia – low-fat milk products

Introduction

Elevated LDL cholesterol is a major risk factor for cardiovascular disease. The serum LDL cholesterol level can be lowered through the reduction of cholesterol absorption or through synthesis. Plant sterols, which resemble cholesterol chemically, have been shown to inhibit the absorption of dietary and biliary cholesterol in the gut [1]. Foods enriched with plant sterols or their saturated form, stanols, are recommended for people

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with mild hypercholesterolemia and for those receiving statin therapy to increase the LDL cholesterol-lowering effectiveness. Plant sterols and stanols are often esterified because in this form they are easily soluble in various food matrices, especially in fat-based foods. Dietary esterified plant sterols and stanols in doses of ≥ 2 g/d, added to an oily food matrix such as margarine, have consistently been shown to lower serum LDL cholesterol by 10–15% [2, 3].

The cholesterol-lowering effect of high-fat spreads enriched with plant sterol or stanol esters on hyperlipidaemic subjects has been widely studied. However, the effects of low-fat products have been less explored. In most studies, low-fat milk products enriched with sterol or stanol esters have reduced LDL cholesterol efficiently [4–8]. However, at least in two studies the LDL cholesterol-lowering effect was weakened when provided as low-fat of non-fat foods and beverages [9, 10]. The mechanism of the action of the cholesterol-lowering effect of plant sterols or stanols is linked to competition with cholesterol for uptake into the dietary mixed micelles. Therefore bile flow, which is triggered by food consumption and by the energy and/or fat intake of a meal, plays a crucial role. It has been reported that the ingestion of a plant sterol-enriched single-dose yoghurt drink with a meal, compared to the same product without a meal, adds significantly to its LDL cholesterol-lowering effectiveness [5]. In one study, the frequency of the ingestion of the plant stanols did not affect its efficiency [11], but this issue merits further investigation. As many as 20–23% of the hypercholesteraemic subjects have revealed non-responsiveness to the plant-sterol-enriched milk [12]. Large inter-individual differences are also seen in LDL cholesterol absorption responses to the plant-sterol-enriched milk [13].

In most earlier studies of the cholesterol-lowering efficacy of low-fat milk products enriched with plant stanols or sterols, the subjects have been moderately hypercholesteroleamic with a total cholesterol of 5.0–8.0 mmol/l and mean LDL cholesterol of ≥4.0 mmol/l [5, 7, 8, 12, 14]. As far we know, there has only been one study with low-fat milk products in which the LDL baseline values were <3.5 mmol/l with daily consumption of 2 g of plant stanol ester, the amount normally added in commercially available supplemented foods [15]. It is important to study subjects with milder hypercholesterolemia, with a total cholesterol of 5.0–6.5 mmol/l and mean LDL cholesterol of about 3.5 mmol/l, since these make up a large proportion of the consumers of low-fat products enriched with plant stanols. Other factors which may modify the effectiveness of the esterified low-fat milk products (e.g., occasion/frequency of ingestion) also merit further study. The purpose of this study with 199 hypercholesterolemic subjects was to investigate the effects of three different low-fat milk products (yoghurt, a yoghurt single-shot drink, or milk) enriched with plant stanol esters on serum lipids.

Materials and methods

Subjects

The present study consisted of four sub-studies of three low-fat milk products: (1) yoghurt (n = 60), (2) a yoghurt single-shot drink, Study I (n = 61), (3) a yoghurt single-shot drink, Study II (n = 19), and (4) milk (n = 59). In all four, the inclusion criteria were: serum total cholesterol concentration of between 5.0 and 6.5 mmol/l at screening, and total triacylglycerol concentration of below 3.0 mmol/l; age 25–65 years; no lipid-lowering medication; no unstable coronary artery diseases. Subjects with diabetes mellitus, malignant disease, alcohol abuse, milk allergy, and pregnancy were also excluded. The baseline characteristics are presented in Table 1. There were no differences between the groups in baseline gamma-glutamyltransferase, creatinine, glucose, uric acid, or lipoprotein B concentrations.

The Ethics Committee of the Department of Medicine, Helsinki University Central Hospital, approved the study protocol of each of the four sub-studies. The yoghurt study was carried out in spring 2003, the yoghurt single-shot drink Study I in autumn of the same year, Study II in spring 2004, and the milk study the following autumn.

Study design

All four sub-studies had a randomized, placebo-controlled, double-blind parallel design with an intervention of 5 weeks. During the intervention period the subjects received either a low-fat milk product (yoghurt, yoghurt single-shot drink or milk) enriched with plant stanol esters, or one of these three products without the plant stanol esters (control group). In the stanol group, the target intake of plant stanols was

Table 1 Baseline characteristics of the stanol (n = 103) and control (n = 99) groups. Results are given as mean ± SD (range) for the combined data of the four sub-studies

	Stanol	Control
Age, years	46.7 ± 11.0	46.4 ± 10.4
BMI (kg/m^2)	24.9 ± 3.3	25.5 ± 3.6
Total cholesterol, mmol/l	5.7 ± 0.1	5.8 ± 0.1
HDL cholesterol, mmol/l	1.8 ± 0.0	1.7 ± 0.0
LDL cholesterol, mmol/l	3.4 ± 0.1	3.5 ± 0.1
Triglycerides, mmol/l	1.2 ± 0.1	1.3 ± 0.1
Male	32 (31%)	41 (41%)

Table 2 The nutrient contents per 100 g of the yoghurt, yoghurt single-shot drink and milk, which included the fatty acid part of the plant stanol esters, and the nutrient contents per 100 g of the placebo yoghurt, yoghurt single-shot drink and milk

	Energy (kJ)	Protein (g)	Carbohydrates (g)	Fat (g)
Plant stanol enriched				
Yoghurt	310	3.4	13	0.9
Yoghurt single-shot drink	330	3.4	13	1.4
Milk	180	3.3	4.8	1.3
Placebo				
Yoghurt	260	3.0	12	0.1
Yoghurt single-shot drink	300	3.9	14	0.1
Milk	170	3.3	4.8	1.0

2 g/d. The nutrient contents per 100 g of the products are shown in Table 2. The daily amount of low-fat milk product ingested was 150 ml in the yoghurt study, 100 ml in the yoghurt single-shot drink (Studies I and II), and 500 ml in the milk study. During the 5-week intervention, the subjects were advised to consume the whole daily amount of the study product with a meal or meals. In the yoghurt single-shot drink Study II, lunch was even provided for the participants in order to ensure that the study product was ingested with that meal, which was consumed under supervision at the staff canteen. The nutrient content recommendations' for a normal workplace followed the Finnish recommendations for a workplace lunch (E% = percentage of the total energy content): 30 E% of fat, 15 E% of proteins, 55–60 E% of carbohydrates; ferrin 2.1 per MJ of energy, vitamin C 8 mg per MJ of energy and vitamin A 105 μg per MJ of energy [16]. The plant stanol ester mixture contained sitostanol and campestanol, which were transesterified with rapeseed oil fatty acids (Raisio plc, Helsinki, Finland). The subjects were not allowed to consume any additional plant-stanol- or plant-sterol-enriched products for 3 weeks before the study or during the study itself. The subjects were asked to maintain their normal diet. They were interviewed about their physical activities, smoking habits, and lifestyle by means of a structured questionnaire to find out whether there were any major changes which might affect the results of the study. Medication and changes in medication were noted at each of the five visits. Weight was measured with digital scales at every visit. During the two last study visits, adverse effects were noted by using a structured questionnaire about the occurrence and severity of any possible gastrointestinal or other symptoms.

Laboratory measurements

Blood samples were collected at each visit after a 12-h overnight fast: two baseline measurements (week −1 and week 0) and at weeks 3, 4, and 5 during the intervention period. Serum total cholesterol, HDL cholesterol, and triacylglycerol concentrations were determined at each visit. Estimated LDL cholesterol was calculated by the Friedewald equation [17]. Gamma-glutamyltransferase, creatinine, glucose, uric acid, and lipoprotein B concentrations were determined at the second study visit (week 0) and at the final one (week 5).

Statistics

In all the sub-studies, serum LDL cholesterol was the primary outcome variable. Serum total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol were determined 5 times during the study (weeks −1, 0, 3, 4, and 5). For each sub-study, the mean of measurements before the intervention (weeks −1 and 0) was defined as the baseline. The changes from baseline to the last two measurements (mean of weeks 4 and 5) were calculated as mmol/l and as percentages. The *t*-test for independent samples was used to compare the difference in these changes between the stanol group and the control group. The treatment differences are given as mean (95% CI) for each sub-study separately. The results of the sub-studies were then pooled using fixed effects models [18], after consideration of heterogeneity between the sub-studies. The mean difference between stanol and placebo was calculated with the standard error of the difference for each sub-study. The pooled treatment difference was calculated using the standardized mean treatment difference weighted by the inverse of the variance of the difference for each study. The pooled standard error and the corresponding 95% confidence interval were calculated. The overall treatment difference was tested using the Z statistic. Heterogeneity was tested using an χ^2 test (Q statistic), with *p*-values of less than 0.10 representing significance.

We completed a sensitivity analysis of the baseline LDL level (<3.5 mmol/l and ≥3.5 mmol/l) only for the primary outcome variables. In addition, we studied the effect of stanol on total cholesterol and LDL cholesterol after 3, 4, and 5 weeks of treatment. The statistical analyses were performed using the SPSS for WINDOWS (version 13.0; SPSS Inc, Chicago, IL).

Results

Subject characteristics

Data from 199 mildly hypercholesterolemic subjects showed no significant changes in body weight during the 5-week intervention period in either the control or

Table 3 Change (%) in serum lipid concentrations from baseline (mean of weeks −1 and 0) to the end of the intervention (mean of weeks 4 and 5). Results are given for the four sub-studies (yoghurt, yoghurt single-shot Studies I and II, and milk) and the meta-analysis of the studies. The meta-analysis used a fixed-effect statistical model

	Study[a]	Stanol (n = 102)		Control (n = 97)		Stanol versus Control		p	Weight (%)
		Mean	SEM	Mean	SEM	Mean	95% CI		
Total	Yoghurt	−2.7	1.6	0.6	1.4	−3.3	−7.6 to 1.0		26.0
Cholesterol	Yoghurt SS I	−0.4	1.6	3.4	1.3	−3.8	−7.9 to 0.3	*	27.6
	Yoghurt SS II	−4.3	2.8	3.8	3.2	−8.1	−17.0 to 0.8	*	6.5
	Milk	−0.9	1.1	2.6	1.3	−3.5	−6.9 to −0.1	**	39.8
	Total					−3.8	−6.0 to −1.7		
Test for heterogeneity: $\chi^2 = 1.115$, df = 3, $p = 0.773$									
Test for overall effect: $Z = 3.55$, $p < 0.001$									
LDL	Yoghurt	−2.9	2.3	0.0	2.2	−2.9	−9.2 to 3.4		24.6
Cholesterol	Yoghurt SS I	−0.3	2.4	2.8	1.8	−3.2	−9.0 to 2.8		27.2
	Yoghurt SS II	−10.6	4.6	1.2	4.5	−11.8	−25.5 to 1.8	*	5.9
	Milk	−3.4	1.6	2.8	1.8	−6.2	−11.0 to −1.5	**	42.4
	Total					−4.9	−7.9 to −1.8		
Test for heterogeneity: $\chi^2 = 2.238$, df = 3, $p = 0.525$									
Test for overall effect: $Z = 3.14$, $p = 0.002$									
HDL	Yoghurt	−3.7	1.5	−2.3	1.8	−1.4	−6.1 to 3.3		27.3
Cholesterol	Yoghurt SS I	0.7	1.6	3.2	1.6	−2.5	−7.1 to 2.0		29.4
	Yoghurt SS II	3.7	1.6	0.4	2.9	3.3	−4.0 to 10.6		13.3
	Milk	3.8	1.5	0.6	1.7	3.2	−1.3 to 7.7		30.0
	Total					+0.3	−2.1 to 2.7		
Test for heterogeneity: $\chi^2 = 4.581$, df = 3, $p = 0.205$									
Test for overall effect: $Z = -0.22$, $p = 0.826$									
Triacylglycerol	Yoghurt	16.7	7.4	14.8	4.6	1.9	−15.9 to 19.7		19.9
	Yoghurt SS I	1.7	4.7	10.7	4.6	−8.9	−22.1 to 4.2		35.2
	Yoghurt SS II	13.8	7.6	26.4	9.9	−12.6	−38.7 to 13.4		9.7
	Milk	−1.0	3.5	1.3	5.6	−2.4	−15.1 to 10.4		35.2
	Total					−4.8	−12.5 to 2.8		
Test for heterogeneity: $\chi^2 = 1.514$, df = 3, $p = 0.679$									
Test for overall effect: $Z = 1.14$, $p = 0.216$									

[a]yoghurt (n = 60, 31 in stanol group + 29 in control group), yoghurt single-shot drink Study I (n = 61, 29 + 32), yoghurt single-shot drink Study II (n = 19, 10 + 9) and milk (n = 59, 32 + 27)
*$p \leq 0.10$, **$p \leq 0.05$

the stanol group. There were no significant changes in dietary intake or physical activity (data not shown), or in the safety parameters during the intervention or between the two groups (data not shown).

Plasma lipid profile in response to treatment

Results of the four sub-studies

The treatment effects (stanol group versus control group) in the four sub-studies are presented in Table 3. The treatment differences for total cholesterol varied between −8.1% and −3.3% in the stanol group compared to the control group. The sub-study with milk yielded a significant treatment effect. For LDL cholesterol, the treatment differences varied between −11.8% and −2.9%, and the sub-study with milk found a statistically significant difference between the groups.

Meta-analyses of the four sub-studies

The results of the meta-analyses are presented in Table 3. The tests for heterogeneity were nonsignificant for all the outcome variables shown in Table 3. Stanol treatment reduced total cholesterol concentration by 3.8% (95% CI 1.7–6.0%, $p < 0.001$) or by 0.23 mmol/l (0.11–0.35 mmol/l, $p < 0.001$). For the LDL-cholesterol concentration, the pooled treatment difference was −4.9% (−7.9 to −1.8%, $p = 0.002$) or −0.19 mmol/l (−0.30 to −0.08 mmol/l, $p < 0.001$), when the stanol treatment was compared to the control. Stanol had no significant effect on HDL cholesterol or triacylglycerol concentrations. The pooled treatment difference was 0.3% (−2.1 to 2.7%, $p = 0.826$) or 0.003 mmol/l (−0.04 to 0.04 mmol/l, $p = 0.884$) for HDL and −4.8% (−12.5 to 2.8%, $p = 0.216$) or −0.06 mmol/l (−0.17 to 0.04, $p = 0.244$) for triacylglycerol concentration.

Stanol had no significant effect on gamma-glutamyltransferase, creatinine, glucose, or uric acid. The changes from baseline were analyzed and the pooled treatment difference was 2.2% (95% CI −3.1 to 7.5%, $p = 0.407$) for gamma-glutamyltransferase, 1.5% (−0.6 to 3.7%, $p = 0.154$) for creatinine, −0.5% (−2.5 to 1.4%, $p = 0.586$) for glucose and 1.3% (−2.9 to 5.4%, $p = 0.552$) for uric acid. The heterogeneity between sub-studies was also non-significant.

Sensitivity analysis

The sensitivity analysis showed that the treatment difference was strongly associated with the LDL baseline. LDL cholesterol decreased relatively more in subjects with an LDL baseline of ≥ 3.5 mmol/l (pooled treatment difference −8.4% (−12.3 to −4.5%, $p < 0.001$) or −0.32 mmol/l (−0.48 to −0.17 mmol/l, $p < 0.001$)) compared to those with an LDL baseline of <3.5 mmol/l (pooled treatment difference −2.5% (−6.7 to 1.6%, $p = 0.232$) or −0.1 mmol/l (−0.2 to 0.0 mmol/l, $p = 0.176$)). Significant pooled treatment differences in total cholesterol and LDL cholesterol were already seen after 3 weeks of treatment (Fig. 1).

There were no significant differences in response between the stanol ester and the control group in apolipoprotein B concentrations. However, the tendency was consistent with the LDL cholesterol responses: the mean decrease in apolipoprotein B was 2.5% in the stanol ester group and 0.8% in the control group. The pooled treatment difference was −2.4% (−6.0 to 1.2%, $p = 0.193$) when the stanol was compared to the placebo.

The treatment effect was not associated with gender, age, weight, or body mass index.

Discussion

In the present randomized, placebo-controlled, double-blind study of 199 moderately-hypercholesterolemic subjects, a daily intake of low-fat milk products (yoghurt, a yoghurt single-shot drink, or milk) enriched with 2 g of plant stanols (delivered as the ester), significantly lowered total cholesterol and LDL cholesterol compared to the respective control product without stanol. There were four sub-studies with different sample sizes, so a meta-analysis was performed to increase the statistical power for the primary end-point and to achieve a more precise estimate of the effective size of plant stanol esters.

The LDL cholesterol-lowering effect of margarine enriched with plant sterol or plant stanol esters has been shown in earlier studies to be between 8 and 15% [19–21], reducing coronary heart disease incidents by

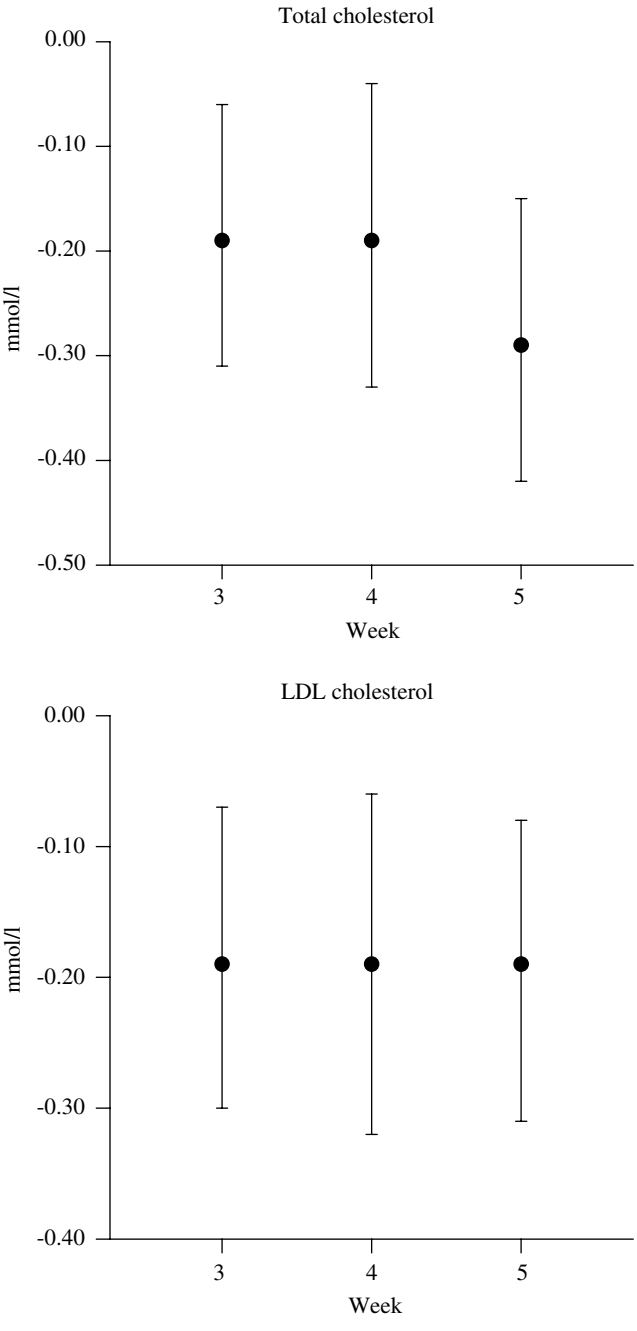

Fig. 1 Meta-analyses of treatment differences (stanol versus control) of the change (mmol/l) from baseline to weeks 3, 4, and 5 in total cholesterol and LDL cholesterol. Dots represent point estimates, and vertical lines denote 95% confidence intervals

15–20% over a period of 5 years [22]. In a meta-analysis by Katan et al. [3], stanols reduced LDL cholesterol by 10.1% while sterols reduced LDL cholesterol by 9.7% though the difference was non-significant. There have been a few controlled studies on the LDL responses to plant sterol- or stanol esters enriched low-fat milk products such as a yoghurt single-shot drink (which

reduced LDL by 5–12%) [4, 5], yoghurt (which reduced LDL by 5–13.5%) [6–8, 15], and milk (which reduced LDL by 8–15.5%) [7, 8]. The wide variety seen in the LDL responses may be accounted by the different doses of stanols/sterols (1.6–3.0 g/day), the esterification of stanol/sterols, the ingestion of the stanols/sterols with or without a meal, and the genotype of the subjects. In the present study, the LDL cholesterol responses were low percentagewise compared to some earlier results [6, 7], but in line with the results of the low-fat yoghurt studied by Noakes et al. [8].

Focussing on the dose response of the cholesterol-lowering effect of plant sterols or stanols, a continuous dose-dependency appears in doses of up to 2 g [23]; larger doses of up to 3–4 g slightly enhance the effect [2]. However, in a recent study by Clifton et al. [7], only 1.6 g/day of phytosterols in the form of sterol esters in yoghurt and milk reduced total and LDL cholesterol by 8.6% and 16%, respectively. One explanation for the different responses may be a baseline LDL cholesterol value of 4.3 mmol/l [7] as compared to 3.4 mmol/l in our study. An important finding of this study was that the treatment difference was strongly associated with the LDL baseline. Differences expressed as percent change were −8.4% with baseline LDL-cholesterol concentrations >3.5 mmol/l and −2.5% with baseline LDL-cholesterol of < 3.5 mmol/l. The effect of the LDL baseline levels on treatment responses is supported by Mussner et al. [24] but not by other studies [8, 19, 25, 26].

The frequency of consumption of low-fat milk products enriched with plant stanol esters is another factor that requires investigation. In our study, the yoghurt and yoghurt single-shot drink were consumed once a day, whereas the 500 ml of milk was ingested by most of the subjects in 2–3 portions with their meals. The frequency of consumption has varied in earlier studies between once [4, 5], twice [7] or three times a day [6], or without restriction [8]. The results of Plat et al. [11] demonstrated that the efficacy of plant stanols in margarine was same, irrespective of the frequency of consumption. As, to our knowledge, only one article [11] has been published that compares the effects of the frequency of consumption of plant stanols, this issue merits further investigation.

In all four sub-studies, the subjects were advised to ingest the study product with lunch, since plant stanol esters inhibit the absorption of both dietary and biliary cholesterol. The yoghurt single-shot drink, Studies I and II, differed in that in Study II lunch was provided for all the participants to ensure that the study product was ingested with a meal. This may be an important explanatory factor for the more pronounced results from yoghurt single-shot drink Study II compared to Study I (treatment effect 11.8% vs. 3.2%). Yoghurt single-shot drinks are generally ingested between meals, thus it is important to investigate whether the responses differ with the time of ingestion. A recent paper by Doornbos et al. [5] also shows that the response to the yoghurt single-shot drink was enhanced when it was ingested with a meal. The LDL cholesterol-lowering efficacy of plant stanol esters is dependent on intestinal micelle formation, since the plant stanol esters compete with cholesterol for incorporation into mixed micelles [27]. Thus it is preferable to consume low-fat products enriched with stanol esters with meals. Solid meals delay gastric emptying and thus prolong the time for micelle formation. The workplace lunch had a fat content of about 30 E% (20–30 g), which ensures the initiation of the bile secretion flow and of micelle formation.

According to earlier studies, the ingestion of plant stanol esters reduced serum LDL cholesterol levels significantly within a few weeks [6, 7, 28]. In the present study, the plant stanol esters reached their maximal reduction by the time of the first measurement in the experimental period, 3 weeks after the initiation of the treatment. Small and non-significant apolipoprotein B changes were in parallel with the LDL concentration, in line with earlier study [29]. Apolipoprotein B is considered to be an even better predictor of coronary artery disease than LDL cholesterol, since it indicates the number, not the size or composition, of LDL particles.

Two of the sub-studies were carried out in the spring (yoghurt study and yoghurt single-shot drink Study II) and two in the autumn (yoghurt single-shot drink Study I and milk study). Seasonal variations in LDL cholesterol [30–32] affected the results of our study slightly, since the LDL cholesterol levels rose about 3% in the control groups of the sub-studies carried out in the autumn.

In conclusion, low-fat milk products (yogurt, yoghurt single-shot drink or milk) enriched with plant stanol esters lowered both total cholesterol and LDL cholesterol. In our study, the test products lowered serum cholesterol less than in those studies in which plant stanol or sterol esters were incorporated in fat-based foods such as margarine or spreads; however, compared to the control group, the difference was highly significant. The effect tended to be more pronounced when the stanol esters were ingested with meals, and when the LDL baseline level was 3.5 mmol/l or higher.

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