# Plant sterol ester–enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia[1,2]

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**ABSTRACT**

**Background:** Naturally occurring plant sterol esters (SEs) favorably affect serum cholesterol concentrations in humans and could aid in the treatment of children with familial hypercholesterolemia (FH).

**Objective:** We studied the effect of SE-enriched spread on serum lipids, lipoproteins, carotenoids, fat-soluble vitamins, and physiologic variables in children with FH aged 7–12 y.

**Design:** In a randomized, double-blind crossover study comprising two 8-wk interventions, 38 children with FH consumed 18.2 ± 1.5 g SE spread/d, corresponding to 1.60 ± 0.13 g SEs, or a control spread. Blood samples were analyzed at the start and end of each diet period.

**Results:** Plasma LDL-cholesterol concentrations decreased by 10.2% ($P = 0.003$) during the SE period compared with the control period. Total cholesterol and apolipoprotein B concentrations were reduced by 7.4% ($P = 0.007$ and $P = 0.020$, respectively) during the SE period. No changes were observed in HDL cholesterol, triacylglycerol, or apolipoprotein A-I. Serum concentration of lipid-adjusted lycopene decreased by 8.1% ($P = 0.015$) in the SE period, with no changes in the other carotenoids. Lipid-adjusted retinol and α-tocopherol concentrations increased by 15.6% ($P < 0.001$) and 7.1% ($P = 0.027$), respectively. There was an increase (16.8%, $P = 0.04$) in alanine transaminase in the SE period, but this was explained by a significantly lower starting concentration in the SE period than in the control period. The children consumed a recommended American Heart Association Step I diet during both intervention periods.

**Conclusion:** A daily intake of 1.6 g SEs induces an additional reduction in LDL-cholesterol concentrations in children with FH consuming a recommended diet. *Am J Clin Nutr* 2002; 76:338–44.

**KEY WORDS** Plant sterol ester, plant sterol supplement, sitosterol ester, familial hypercholesterolemia, serum cholesterol, children, LDL cholesterol

## INTRODUCTION

Familial hypercholesterolemia (FH) is associated with an increased risk of premature atherosclerosis and coronary artery disease (CAD). The elevated LDL-cholesterol concentrations observed in FH patients are caused by a mutation in the LDL receptor gene (1). Clinical trials showed conclusively that lowering serum cholesterol reduces morbidity and mortality from CAD in patients with established CAD (2) and also reduces new CAD events and mortality in patients without established CAD (3, 4). Moreover, it is generally agreed that FH patients need aggressive lipid-lowering therapy (5). Consensus panels from several Western countries recommend that prevention of CAD should be initiated in childhood. Treatment with bile acid–binding resins has been used successfully in children. However, compliance with use of the resins, which have a sandy consistency after being mixed in fluid, is often poor (6, 7). Statins are the newest class of lipid-lowering drugs. However, treatment with statins has not been evaluated for long-term safety in patients younger than 18 y of age. Thus, a dietary approach is most important in the treatment of children with FH. The recommended diet is usually based on a restriction of total and saturated fat and dietary cholesterol intake. Saturated fat should be replaced by unsaturated fat; thus, butter should be replaced by a spread rich in unsaturated fats. In addition to supplying favorable fatty acids, vegetable oil spread may become a good source of plant sterols, or phytosterols, which have been shown to reduce serum cholesterol concentrations (8–13). Plant sterols are naturally occurring plant compounds; the predominant ones are sitosterol, campesterol, and stigmasterol. The corresponding hydrogenated forms are sitostanol and campestanol (14).

Plant sterols are extracted from vegetable oils and esterified before incorporation into spreads. In several clinical studies, the intake of spread enriched with plant sterol esters (SEs) or plant stanol esters was shown to induce a significant serum cholesterol reduction (8–13, 15–17). Dosages of ≈1.5–3 g plant sterols and stanols/d were shown to reduce total serum cholesterol by 8–17% and LDL cholesterol by 9–19%. The plant sterols inhibit cholesterol absorption in the gut, but the exact mechanism is still not fully understood (16, 18–20).

The serum cholesterol–lowering effect of plant sterols and stanols in children with FH was examined earlier by 2 groups (21–24). Significant effects were observed when sitosterol (21) and sitostanol (22) were incorporated in pastils or were consumed as a sitostanol ester–enriched spread (23, 24). To our knowledge,

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**TABLE 1**
Clinical characteristics of the study population[1]

| | Value |
|---|---|
| Age (y) | 10.5 ± 1.7 (7.1–12.8) |
| Weight (kg) | 41.0 ± 13.3 (21.9–82.6) |
| Height (cm) | 147 ± 14 (122–174) |
| Cholesterol (mmol/L) | |
| Total | 7.01 ± 1.26 (4.90–9.00) |
| LDL | 5.39 ± 1.42 (3.10–7.30) |
| HDL | 1.37 ± 0.32 (1.00–2.30) |
| Triacylglycerol (mmol/L) | 0.56 ± 0.22 (0.30–0.90) |

[1] $\bar{x}$ ± SD; range in parentheses. $n$ = 19 M, 22 F.

**TABLE 2**
Fat and sterol composition of the spreads used in the study[1]

| | Control spread | Plant sterol spread |
|---|---|---|
| | *mg/kg* | |
| Fat | | |
| Total | — (35.2) | — (35.0) |
| Saturated | — (23.4) | — (23.8) |
| Monounsaturated | — (29.8) | — (27.7) |
| Polyunsaturated | — (46.0) | — (47.8) |
| Sterols | | |
| Total | 1644 (0.16) | 87 894 (8.79) |
| Cholesterol | 21 (1.28) | 253 (0.29) |
| Brassicasterol | 62 (3.77) | 951 (1.08) |
| Campesterol | 329 (20.01) | 21 394 (24.34) |
| Stigmasterol | 75 (4.56) | 17 804 (20.25) |
| β-Sitosterol | 772 (46.95) | 41 271 (46.96) |
| Other sterols | 385 (23.42) | 6221 (7.07) |

[1] $\bar{x}$; percentages in parentheses.

there are no published data regarding the effect of SEs in spread on blood cholesterol concentrations, carotenoids, fat-soluble vitamins, and physiologic variables in young children with FH.

The aim of the present study was to estimate the potency of a dosage of ≈1.6 g plant sterols/d, given as SE-enriched spread, to reduce plasma concentrations of LDL cholesterol in children with FH and to investigate the influence of SE-enriched spread on carotenoids and fat-soluble vitamins in serum and other physiologic variables in these children.

## SUBJECTS AND METHODS

### Subjects

Forty-one healthy children were recruited from the patient register at the Lipid Clinic at the National Hospital in Oslo. All subjects had a mother or father with hypercholesterolemia and were diagnosed with "definite" or "possible" heterozygous FH (25). The diagnosis was confirmed by documentation of the presence of an FH mutation in 25 of the children. In the rest of the subjects, the mutations were still unknown. Clinical characteristics of the subjects are presented in **Table 1**. All the children were healthy and had no clinical symptoms of hypercholesterolemia (ie, tendon xanthomata, corneal arcus, xanthelasmata). Three of the boys used cholesterol-lowering resins (colestipol, trade name Lestid, Pharmacia & Upjohn, New Jersey) but stopped taking the drugs 8 wk before random assignment. Four subjects used drugs against allergy or asthma problems. Thirteen children took fish oil supplements (cod-liver oil or n−3 supplements), and 8 children took multivitamin supplements containing vitamins A, D, and E during the study. The subjects were instructed to take the same dosages of medication or supplements during the entire study period. Three of the girls reached menarche before the start of the study and one during the first intervention period. Written, informed consent was given by the parents, and the study was approved by the Regional Committee for Medical Research Ethics, Southern Norway.

### Experimental design

The study had a randomized, double-blind crossover design comprising two 8-wk intervention periods. Preceding the random assignments, there was a run-in period of 3 wk, followed by an intervention period of 8 wk, 4 wk of washout, another 2 wk of run-in, and a second intervention period of 8 wk.

The first run-in period was included to check compliance and to standardize the spread intake. The subjects were given the control spread in small tubs of 20 g. A compliance rate of 50% was required for the subjects to continue in the study. At the start of the first intervention period, the subjects were randomly assigned to eat 20 g SE-enriched spread or control spread/d for 8 wk and were switched to the other spread in the second intervention period.

A physical examination and a history of disease were undertaken on day 1. Blood samples were drawn for analyses of baseline plasma lipids on day −21. On days 1 and 56 in each intervention period, blood samples were drawn for analyses of lipids and safety factors. Body weight was measured on days 1 and 56 and height was measured on day 1 in each period.

The children and their parents were seen 8 wk before the random assignments, and dietary information was given to groups of 4–8 families. All the families had previously received detailed dietary information and made changes according to the recommendations. The subjects and their families were advised to reduce the intake of saturated fat and cholesterol and to increase the intake of unsaturated fat, fruit, and vegetables. Practical instructions were given about how to choose the right food products. The families were encouraged to make additional changes and to follow the dietary advice during the entire study. In the middle of each intervention period, a 4-d weighed-food record was kept. The subjects were instructed to weigh and record all food consumed for 4 consecutive days, including one Saturday or Sunday. Digital scales were provided by the clinic. Mean dietary intake was calculated by using the computer software MAT PÅ DATA (version 3.0; LKH, Oslo). A questionnaire about the subjective experience of the amounts and taste of the consumed spread was filled out at the end of the study.

### Administration of spread

The SE-enriched and control spreads were produced and prepared by Unilever BestFoods, Purfleet, United Kingdom and Unilever Research, Vlaardingen, Netherlands, respectively. The compositions of the spreads are presented in **Table 2**. Plant sterols were extracted from soybeans and other vegetable oils such as sunflower oil and esterified with fatty acids from sunflower seed oil to an esterification degree of 85–90%. The SE spread contained 8.8% free plant sterols, of which ≈50% was sitosterol. Twenty grams of the spread supplied 1.76 g plant sterols. The fat and

**TABLE 3**
Dietary intake of the subjects in the 2 diet periods[1]

| Nutrients | Control period | Plant sterol period |
|---|---|---|
| Energy | | |
| (MJ) | 6.9 ± 1.3 | 6.8 ± 1.4 |
| (kcal) | 1651 ± 315 | 1639 ± 328 |
| Fat (% of energy) | | |
| Total | 26.3 ± 3.4 | 25.4 ± 4.7 |
| Saturated | 9.1 ± 1.9 | 8.7 ± 2.4 |
| Monounsaturated | 8.6 ± 1.7 | 8.2 ± 1.9 |
| Polyunsaturated | 5.5 ± 1.1 | 5.5 ± 1.3 |
| Protein (% of energy) | 14.6 ± 2.9 | 14.9 ± 1.9 |
| Carbohydrates (% of energy) | 59.1 ± 4.5 | 59.7 ± 5.3 |
| Cholesterol (mg) | 121 ± 57 | 113 ± 44 |

[1] $\bar{x}$ ± SD; $n$ = 37. No significant differences in energy or nutrient intake were noted between the diet periods.

sterol composition of the spreads was analyzed according to methods described by Weststrate and Mejer (10).

The spread was distributed to the subjects in 20-g tubs, and they were instructed to eat one tub daily. It was preferred that the subjects consume it as a spread on sandwiches, but they could also eat it as part of a hot meal if the spread was mixed with the food on the plate. They were instructed to eat the spread 3 times/d. Each day, the subjects reported the consumption of the spread on a checklist. In addition, all tubs, with and without leftovers, were delivered to the clinic and weighed at the end of the intervention periods. Because some leftovers were expected, 19.8 g of spread (1.7 g plant sterols) corresponded to 100% compliance. In the middle of each intervention period, compliance was reported by one of the parents by telephone or a visit at the clinic.

The tubs were unlabeled and delivered to the subjects twice during each intervention period in boxes of 32 tubs (28 + 4 extra tubs). The boxes were labeled with subject, period, and random assignment number. Packaging and labeling were performed by personnel not involved with the subjects or data handling. All other personnel and the statisticians were blinded to the treatments.

### Analyses

Venous blood samples were obtained after the subjects had fasted 12 h overnight. Plasma total, LDL-, and HDL-cholesterol; triacylglycerol; apolipoprotein (apo) A-I; and apo B concentrations were analyzed from frozen samples at the local laboratory at the National Hospital in Oslo at the end of the study. Plasma total and HDL-cholesterol and triacylglycerol concentrations were analyzed enzymatically (Boehringer Mannheim, Mannheim, Germany) and LDL cholesterol was calculated with the Friedewald equation (26). Apo A-I and apo B were analyzed by immunonephrometry with the use of Behrings instrument and reagents (Dade Behringer, Marburg, Germany).

Serum concentrations of carotenoids, retinol, and α-tocopherol were analyzed from frozen serum samples at the Commonwealth Scientific and Industrial Research Council Organisation in Adelaide, Australia. Serum was collected and stored in light-protected tubes at −70 °C until analyzed. Serum extractions were performed according to the method of Yang and Lee (27), and carotenoids, retinol, and α-tocopherol were determined by HPLC. Minor modifications to this method were derived from Khachik et al (28). A Shimadzu LC 10 HPLC (Shimadzu, Kiyoto, Japan) fitted with a refrigerated autosampler and a SPD-M10Avp photodiode array detector with a class LC 10 chromatography workstation was used for analyses of the prepared samples. The carotenoids were detected at 450 and 472 nm, retinol at 325 nm, and α-tocopherol at 292 nm. Serum carotenoid, retinol, and α-tocopherol concentrations were standardized for plasma lipid concentration (total cholesterol and triacylglycerol) because the lipoproteins are carriers of carotenoids and fat-soluble vitamins.

At the start and end of each intervention period, blood analyses of albumin, alkaline phosphatase (EC 3.1.3.1), calcium, phosphate, uric acid, hemoglobin, erythrocytes, leukocytes, alanine transaminase (ALT; EC 2.6.1.2), aspartate transaminase (EC 2.6.1.1), bilirubin, and creatinine were performed, and the results were evaluated by a physician. Thyroid-stimulating hormone and thyroxin were analyzed only at the start of the study to exclude hypothyroidism as a cause of the hypercholesterolemia. The genetic analyses were performed at the Medical Genetics Laboratory, National Hospital, Oslo, with the method of Leren et al (29).

### Statistics

A power calculation was performed to determine the required number of subjects. A sample size of 26 children would have a power of 90% to detect a difference of ≈10% in LDL cholesterol with $\alpha = 0.05$. Statistical analyses were performed with the SPSS for WINDOWS 6.0 statistics program (SPSS Inc, Chicago). Descriptive data and results are presented as means ± SDs or ranges. Statistical differences between the diet periods were tested with the use of the paired Student's $t$ test. The changes within the diet periods (start − end) were used for comparison of lipid effects and safety factors. Period and carryover effects were investigated by simple regression analysis, and no effects were observed. Regression analysis was also used to analyze the effect of age and sex on the observed lipid changes. A significance concentration of 0.05 was chosen.

## RESULTS

### Exclusions

Two subjects (girls) dropped out during the study because of family problems not related to the project, and a third (girl) because she found that the amount of spread was too large. Thirty-eight subjects (19 girls and 19 boys) completed the study and are included in the final analyses.

### Compliance

The children consumed 90.9% (range: 65–100%) of the control spread and 91.7% (range: 67–100%) of the SE spread. This corresponds to a daily intake of 18.0 ± 1.7 and 18.2 ± 1.5 g of the control and SE spreads, respectively. Thus, the mean intake of plant sterols was 1.60 ± 0.13 g/d in the SE period.

The spread was eaten 3 times/d by 67% of the subjects, 2 times/d by 22%, and 2 or 3 times/d by 11% of the subjects. Thirty-two percent of the subjects expressed the opinion that the amount of spread was too large, but 68% were satisfied with the amounts. Seventeen of 37 subjects (46%) reported that they could recognize a difference between the 2 spreads but could not separate the SE from the control spread. Difference in color was most frequently reported. Of those reporting a difference between the spreads, 56% indicated that the SE spread tasted better than the control spread (blinded question).

### Dietary intake

The 4-d food record was completed by 37 of the subjects, and the mean dietary intake is presented in **Table 3**. There was no significant difference in energy or nutrient intake between the diet periods. The mean intakes of total and saturated fat in the group

**TABLE 4**
Blood lipids and lipoproteins in the 2 diet periods[1]

| | Control period | | Plant sterol period | | | |
|---|---|---|---|---|---|---|
| | Start | End | Start | End | Difference | $P^2$ |
| | | | | | *mmol/L* | |
| Cholesterol (mmol/L) | | | | | | |
| Total | 7.46 ± 1.74 | 7.48 ± 1.70 | 7.38 ± 1.58 | 6.87 ± 1.45 | −0.53 ± 1.14 (−7.4 ± 15.5) | 0.007 |
| LDL | 5.80 ± 1.81 | 5.88 ± 1.79 | 5.76 ± 1.71 | 5.25 ± 1.55 | −0.58 ± 1.12 (−10.2 ± 18.1) | 0.003 |
| HDL | 1.25 ± 0.35 | 1.25 ± 0.31 | 1.23 ± 0.30 | 1.26 ± 0.35 | 0.034 ± 0.27 (1.0 ± 24.8) | NS |
| Triacylglycerol (mmol/L) | 0.91 ± 0.73 | 0.78 ± 0.33 | 0.84 ± 0.43 | 0.80 ± 0.37 | 0.095 ± 0.76 (−1.1 ± 67.1) | NS |
| Apo A-I (g/L) | 1.32 ± 0.24 | 1.35 ± 0.23 | 1.30 ± 0.26 | 1.32 ± 0.26 | −0.024 ± 0.25 (−1.7 ± 20.6) | NS |
| Apo B (g/L) | 1.50 ± 0.43 | 1.48 ± 0.39 | 1.43 ± 0.38 | 1.32 ± 0.35 | −0.095 ± 0.24 (−7.4 ± 17.8) | 0.020 |

[1] $\bar{x}$ ± SD; $n$ = 38. Percentages in parentheses. Apo, apolipoprotein; NS, not significant.
[2] Difference between the changes in the 2 diet periods (two-sided Student's $t$ test, pairwise observations).

were < 30% and 10% of energy, respectively, and cholesterol intake was < 300 mg. Thus, the subjects consumed the recommended American Heart Association Step I diet. Only 1 subject in the control period and 4 subjects in the SE period had a fat intake of > 30% of energy.

### Blood lipids and lipoprotein changes

There was no significant difference between the starting concentrations of plasma lipids in the 2 diet periods (**Table 4**). Plasma LDL-cholesterol concentrations were reduced by 10.2% ($P$ = 0.003) in the SE period compared with the control period. During the SE diet period, the LDL-cholesterol concentration decreased by 7.8%, and an increase of 2.9% was observed in the control period. Total cholesterol was 7.4% ($P$ = 0.007) lower after intake of the SE spread. Plasma total cholesterol decreased by 6.3% and increased by 1.3% in the SE and control period, respectively. The changes in total and LDL cholesterol were not dependent on age or sex. Plasma HDL cholesterol and triacylglycerol did not change significantly after intake of the SE spread. Apolipoprotein B was 7.4% ($P$ = 0.020) lower after the SE period, whereas no changes were observed in apo A-I.

### Safety factors

The changes in safety factors are presented in **Tables 5** and **6**. Because of problems during blood sampling in 1 subject, the data for only 37 subjects are included in Table 5. There was a significant difference in the starting concentrations of ALT and uric acid between the diet periods. The starting concentration of ALT was significantly lower in the SE diet period than in the control period, and this may explain the significant increase in ALT during the SE diet period (16.8%, $P$ = 0.04). None of the subjects had a plasma concentration of ALT outside the reference concentration at any time.

Serum concentrations of fat-soluble vitamins and carotenoids are presented in Table 6. Significant decreases in the serum concentrations of lycopene and β-carotene were observed after intake of the SE spread. After adjustment for lipid changes (total cholesterol and triacylglycerol), however, the differences in β-carotene disappeared, but the lycopene concentration was still 8.1% lower ($P$ = 0.015) after intake of the SE spread compared with the control spread. Serum concentrations of retinol, but not α-tocopherol, were significantly higher in the SE period. After lipid standardization, however, the plasma concentration of retinol was 15.6% ($P$ < 0.001) and that of α-tocopherol 7.1% ($P$ = 0.027) higher in the SE diet period than in the control period. The changes in lycopene and retinol may, at least partly, be explained by significantly higher and lower starting concentrations, respectively, in the SE than in the control period.

## DISCUSSION

In the present study, an intake of 1.6 g plant sterols/d induced an LDL-cholesterol reduction of 10.2% in children with FH.

**TABLE 5**
Changes in safety factors during the study[1]

| | Control period | | Plant sterol period | |
|---|---|---|---|---|
| | Start | End | Start | End |
| Albumin(g/L) | 42.6 ± 2.7 | 42.0 ± 2.1 | 42.8 ± 2.6 | 42.5 ± 2.7 |
| ALT (U/L)[2,3] | 16.4 ± 6.0 | 15.4 ± 5.7 | 14.9 ± 5.4 | 16.3 ± 5.5 |
| AST (U/L) | 18.8 ± 8.1 | 19.1 ± 6.7 | 19.4 ± 7.6 | 19.1 ± 6.1 |
| ALP (U/L) | 534 ± 159 | 557 ± 165 | 543 ± 145 | 550 ± 141 |
| Bilirubin (μmol/L) | 8.2 ± 3.5 | 7.9 ± 2.5 | 7.8 ± 2.5 | 7.5 ± 2.2 |
| Calcium (mmol/L) | 2.4 ± 0.1 | 2.4 ± 0.1 | 2.4 ± 0.1 | 2.4 ± 0.1 |
| Creatinine (μmol/L) | 63.1 ± 7.0 | 63.2 ± 6.1 | 64.3 ± 6.1 | 64.1 ± 7.3 |
| Erythrocytes ($10^{12}$/L) | 4.7 ± 0.3 | 4.8 ± 0.3 | 4.7 ± 0.3 | 4.7 ± 0.3 |
| Hemoglobin (g/dL) | 12.8 ± 0.8 | 12.9 ± 0.8 | 12.8 ± 0.8 | 13.0 ± 0.8 |
| Leukocytes ($10^9$/L) | 5.9 ± 1.5 | 5.5 ± 1.6 | 5.5 ± 1.3 | 5.8 ± 1.9 |
| Phosphate (mmol/L) | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.5 ± 0.1 |
| Uric acid (μmol/L)[3] | 246 ± 42.9 | 240 ± 45.3 | 234 ± 39.2 | 231 ± 37.9 |

[1] $\bar{x}$ ± SD; $n$ = 37. ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase.
[2] Significant difference between the diet periods, $P$ = 0.04.
[3] Significant difference between the starting concentrations in the diet periods, $P$ < 0.05.

**TABLE 6**
Serum concentrations of carotenoids and fat-soluble vitamins in the 2 diet periods[1]

| | Control period | | Plant sterol period | | |
|---|---|---|---|---|---|
| | Start | End | Start | End | $P^2$ |
| Lutein (μmol/L) | 0.41 ± 0.15 | 0.39 ± 0.14 | 0.41 ± 0.16 | 0.37 ± 0.13 | 0.282 |
| Lutein/(TC + TG) (μmol/mmol) | 0.051 ± 0.021 | 0.048 ± 0.018 | 0.051 ± 0.020 | 0.049 ± 0.018 | 0.776 |
| Retinol (μmol/L) | 1.53 ± 0.32 | 1.48 ± 0.30 | 1.44 ± 0.31 | 1.48 ± 0.28 | 0.045 |
| Retinol/(TC + TG) (μmol/mmol) | 0.194 ± 0.065 | 0.188 ± 0.060 | 0.183 ± 0.059[3] | 0.202 ± 0.059 | <0.001 |
| α-Tocopherol (μmol/L) | 36.73 ± 9.23 | 35.21 ± 7.93 | 35.51 ± 7.96 | 34.19 ± 7.29 | 0.858 |
| α-Tocopherol/(TC +TG) (μmol/mmol) | 4.45 ± 0.83 | 4.31 ± 0.72 | 4.36 ± 0.71 | 4.51 ± 0.79 | 0.027 |
| Lycopene (μmol/L) | 0.92 ± 0.43 | 0.94 ± 0.39 | 1.02 ± 0.39 | 0.82 ± 0.29 | 0.004 |
| Lycopene/(TC + TG) (μmol/mmol) | 0.114 ± 0.060 | 0.117 ± 0.050 | 0.128 ± 0.059[4] | 0.109 ± 0.040 | 0.015 |
| α-Carotene (μmol/L) | 0.101 ± 0.091 | 0.092 ± 0.067 | 0.102 ± 0.102 | 0.070 ± 0.047 | 0.159 |
| α-Carotene/(TC + TG) (μmol/mmol) | 0.0124 ± 0.0119 | 0.0113 ± 0.0084 | 0.0124 ± 0.0115 | 0.0093 ± 0.0062 | 0.269 |
| β-Carotene (μmol/L) | 0.63 ± 0.32 | 0.63 ± 0.26 | 0.64 ± 0.29 | 0.52 ± 0.22 | 0.026 |
| β-Carotene/(TC + TG) (μmol/mmol) | 0.0822 ± 0.0553 | 0.0809 ± 0.0410 | 0.0821 ± 0.0483 | 0.0717 ± 0.0381 | 0.108 |

[1] $\bar{x}$ ± SD; $n$ = 38. TC, total cholesterol; TG, triacylglycerol.
[2] Difference between the changes in the 2 diet periods (two-sided Student's $t$ test, pairwise observations).
[3,4] Significantly different from the starting concentration in the control period. [3] $P = 0.038$, [4] $P = 0.043$.

Plasma total cholesterol and apo B both decreased significantly by 7.4%, but no effect was observed in HDL cholesterol, triacylglycerol, and apo A-I. The SE-enriched spread was well tolerated by the children, and no adverse effects in common blood variables were observed. A reduction of 8.1% in serum lycopene was, however, observed. Most of the subjects consumed the spreads in 2 or 3 portions daily.

The present study is, to our knowledge, the first clinical trial of SE-enriched spread in children with FH. Persons with FH are unique in that they have only 50% of the normal number of LDL receptors. Thus, children with FH are born with a higher concentration of circulating cholesterol, and it is of crucial importance to start treatment early in childhood to slow down the atherosclerotic process. Serum cholesterol concentration may be 6–14 mmol/L in heterozygote FH subjects. FH was confirmed by documentation of an FH mutation in 25 of the 38 children in the present study. A total serum cholesterol of >6.7 mmol/L may also be used as a criterion for FH in children aged <16 y (25). In the 13 subjects in whom FH mutations were not discovered, the total cholesterol was <6.7 mmol/L (LDL <5 mmol/L). Thus, some of the included children may not have FH. A negative genetic test was, however, only detected in one subject, but this subject was included in the analyses because of his high plasma cholesterol concentration (6.0 mmol/L). The genetic analysis tested the 19 common mutations of FH. Approximately 62% of the Norwegian FH population has one of these mutations (29). Thus, the 12 children without a positive FH test may have less common mutations or a polygenetic hypercholesterolemia. When the children without confirmed FH were compared with the children with genetically confirmed FH, the baseline total and LDL-cholesterol concentrations were significantly higher in the confirmed FH group (8.1 and 6.6 mmol/L compared with 6.0 and 4.3 mmol/L). There was, however, no statistically significant difference in plasma lipid changes induced by the SE-enriched spread between the 2 groups. The LDL-cholesterol reductions were 0.65 and 0.45 mmol/L in the group with confirmed FH and in the group without confirmed FH, respectively.

Dietary intervention and positive changes in lifestyle are the main recommended treatments in children with FH. Statins are usually not introduced before the age of 18 y in FH patients. The standard dietary advice includes a reduced intake of fat as saturated fat, an increased intake of unsaturated fat, and a reduced cholesterol intake. A strict diet may, however, not result in a decrease in serum cholesterol of >5–20% (30). Thus, additional treatment is needed. In the present study, an additional serum cholesterol–lowering effect was observed when SEs were added to a recommended diet. This is in accordance with studies in adult hyperlipidemic subjects that used SE- or stanol ester–enriched spreads (11, 16, 17, 31, 32). The spreads used in the present study had a high content of mono- and polyunsaturated fatty acids. However, LDL-cholesterol concentrations increased in the control period by almost 3%. This effect may have been caused by changes in dietary habits. This was, however, only reported by 3 of the subjects, who reported an exchange of cereals or fruit for more sandwiches. Because a weight gain was expected in this group of children aged 7–12 y, this outcome cannot exclude the possibility that dietary changes occurred. Approximately 87% of the children were regular spread consumers, although their daily intake of spread was mostly in smaller amounts than were served to them in the study. The recommended type of spread most commonly used by the FH families had high proportions of polyunsaturated (49%) and monounsaturated (24%) fat and was quite similar to the spread used in the trial. Furthermore, the run-in periods were introduced to stabilize the fatty acid intake before the intervention. No difference was observed in nutrient intake between the 2 diet periods. The limitations of a 4-d food record in detecting differences may have to be taken into consideration.

An LDL-cholesterol-lowering effect of ≈10% was reported in previous studies of SE-enriched spreads (10, 12, 16, 17). Usually larger amounts of plant sterols were used. In the study by Hendriks et al (12), 1.61 g plant sterols was given to mildly hypercholesterolemic subjects and an LDL reduction of 8.5%, or 0.26 mmol/L, was observed. Furthermore, a review by Law (13) concluded that an intake of 2 g plant sterols/d may induce an LDL-cholesterol-lowering effect of 0.54 mmol/L in adults aged 50–59 y. The 10.2% reduction in the present study corresponds to a decrease in LDL cholesterol of 0.58 mmol/L. Thus, children with FH may respond to plant sterol intakes at least as well as do adults with normal blood lipids or hypercholesterolemia.

In vitro and in vivo studies have investigated the safety of β-sitosterol and mixtures of SEs without finding significant adverse effects (33–35). No adverse effects were detected in

common blood variables after 8 wk of spread consumption in FH children. Changes in fat-soluble vitamins and carotenoids were reported by several investigators using plant sterol– or stanol ester–enriched spreads (10, 12, 17). In most studies, a decrease in serum carotenoids was observed, but after lipid standardization, the decrease disappeared or diminished. Fat-soluble vitamins and provitamins are transported by the various lipoprotein fractions. Thus, changes in serum lipid concentrations affect the absolute concentrations of the fat-soluble vitamins and provitamins. In the present study, only lipid-adjusted lycopene concentrations were lower (8.1%) after SE-spread intake. This may partly be explained by a higher starting concentration of lycopene in the SE than in the control diet period. In the study by Hendriks et al (12), 1.6 g plant sterols had no effect on lipid-standardized plasma α- and β-carotene or -lycopene, but lower and higher dosages of plant sterols reduced their plasma concentrations.

In contrast with earlier studies, an increase in plasma concentrations of lipid-adjusted retinol and α-tocopherol was observed in the present study. Some of the increase in retinol concentration may be explained by a regression toward the mean because the starting concentration was lower in the SE than in the control period. The content of fat-soluble vitamins in the 2 spreads was the same, but changes in the background diet are a possible explanation for the increase. No difference was observed, however, between the 4-d food record periods, and the crossover design made it unlikely for a seasonal variation in food intake to explain this. Those children taking vitamin supplements and cod-liver oil with vitamins A, D, and E may have been more consistent in their use during the SE diet period. Norwegians have a tradition of consuming cod-liver oil during the winter months. The study was initiated in August, with the first intervention period between October and December and the second between January and March. Either the children in the SE group may have consumed more cod-liver oil than did the control group during the winter months in period 2, or the whole group consumed more cod-liver oil during the SE periods.

The small decrease in serum lycopene observed in the present study is of minor biological and clinical importance. Because of a higher starting concentration in the SE diet period, the actual decrease is probably lower than 8%. When plant sterol supplements are introduced as part of a lipid-lowering diet in children with FH, an increased intake of fruit and vegetables is recommended to compensate for a possible decrease in serum lycopene.

In conclusion, this study shows that in children with FH consuming a recommended diet, a daily intake of 1.6 g plant sterols as SE-enriched spread induces an additional reduction in LDL cholesterol of ≈10% without adverse effects. SE-enriched spread may be an effective and safe tool in the treatment of serum cholesterol in children with FH.

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