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# Effect of a plant stanol ester-containing spread, placebo spread, or Mediterranean diet on estimated cardiovascular risk and lipid, inflammatory and haemostatic factors

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**KEYWORDS**
Mediterranean diet;
Plant stanol ester;
Cardiovascular risk;
Primary prevention;
Hypercholesterolaemia

**Abstract** *Background and aims:* Mediterranean diet is associated with a reduced risk for cardiovascular disease (CVD). Use of plant stanols decreases low density lipoprotein cholesterol (LDL-C) concentrations. We compared the effects of the Mediterranean diet and plant stanol esters on vascular risk factors and estimated CVD (eCVD) risk.
*Methods and results:* In this prospective, randomized, placebo-controlled study, 150 mildly hypercholesterolaemic subjects were randomized to Mediterranean diet, a spread containing plant stanol esters (2 g/day) or a placebo spread. Vascular risk factors were assessed every month for 4 months and the eCVD risk was calculated using the PROspective- Cardiovascular-Munster (PROCAM), Framingham, and Reynolds risk engines. Placebo had no significant effect on risk factors or eCVD risk. Mediterranean diet gradually induced a significant reduction in total cholesterol (TC), LDL-C, triglycerides, high sensitivity C-reactive protein (hsCRP), blood pressure and eCVD risk (24–32%). The plant stanol ester spread reduced (by 1 month) TC (−14%), LDL-C (−16%), hsCRP (−17%), and estimated CVD risk (26–30%). eCVD risk reduction was sustained at 4th months when the gradual Mediterranean diet eCVD risk reduction became comparable to that of the stanol group.

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*Conclusions:* Plant stanol esters yielded an early, by 1st treatment month, reduction of eCVD risk that resulted from a TC, LDL-C, and hsCRP decrease. eCVD risk reduction on the Mediterranean diet resulted from a change in several CVD risk factors and equaled that of plant stanol at 4 months. The consumption of plant stanol esters by moderately hypercholesterolaemic patients may be a useful option to reduce CVD risk in those who do not adopt a Mediterranean diet.


## Introduction

The Mediterranean diet is associated with a reduced risk for type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), cancer and all-cause mortality [1—3]. It was estimated that Mediterranean diet combined with regular physical activity and smoking cessation is associated with an 80% risk reduction for myocardial infarction, 70% for stroke and 90% for T2DM [4]. Therefore, this dietary pattern is attractive as a preventive strategy in both the general population and in patients with established CVD. However, the Mediterranean diet is not adopted by the majority of people in Western countries [3,5]. There is also a decreasing adherence to the Mediterranean diet even in European Mediterranean countries (e.g. Greece, Italy, Spain and Portugal) partly because of practical reasons (e.g. urbanisation, more working women with little time to cook, way of living, type of work as well as socioeconomic and educational reasons) [1,3,5—9].

The National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III report [10] recommends that plant stanols/sterols (2 g/day) should be incorporated into a diet aiming at low density lipoprotein cholesterol (LDL-C) lowering by 10—15%. This LDL-C reduction may make it possible to avoid statins in mildly hypercholesterolaemic patients or reduce their doses [11]. Plant sterol/stanol chemical structure, absorption and origin are described in Fig. 1. They replace cholesterol in intestinal micelles and subsequently less cholesterol is absorbed; increased plant stanol concentrations within the enterocyte also activate cholesterol efflux through the ATP binding cassette A1 system back into the intestinal lumen [12]. In a recent study [13] the reductions in TC and LDL-C were partly associated with dietary enrichment with plant sterols (estimated 220 mg/day) coming from olive oil or nuts [13].

However, we are not aware of any studies comparing the effects of the Mediterranean diet and plant stanol ester consumption on estimated CVD (eCVD) risk in a Mediterranean population. This study was undertaken to answer this question.

## Patients and methods

1. Number of participants-inclusion criteria: We studied 150 patients with mild hypercholesterolaemia [total cholesterol (TC) = 200—250 mg/dl (5.2—6.4 mmol/l)].
2. Study design: Prospective, randomized, placebo-controlled, intension-to-treat study. During an initial

**Occurrence in natural diet**

| Food | Plant sterols [mg/100 g edible portion] |
|---|---|
| Corn oil | 952 |
| Sunflower oil | 725 |
| Safflower oil | 444 |
| Soybean oil | 221 |
| Olive oil | 176 |
| Almonds | 143 |
| Beans | 76 |
| Corn | 70 |
| Wheat | 69 |
| Palm oil | 49 |
| Lettuce | 38 |
| Banana | 16 |
| Tomato | 7 |

**Absorption**

| | Absorption [%] |
|---|---|
| Sitostanol | 0.04 |
| Sitosterol | 0.51 |
| Campestanol | 0.16 |
| Campesterol | 1.90 |
| Cholesterol | 56.0 |

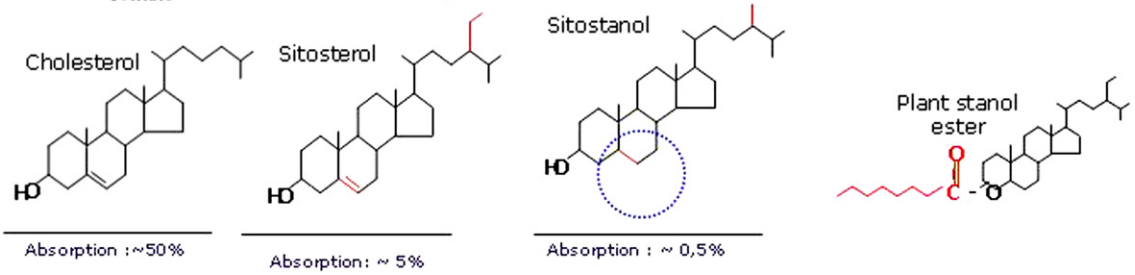


**Figure 1** Chemical composition, absorption and origin of sterols-stanols.

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4-week run-in period, patients were advised by a trained dietician to follow a step I hypolipidemic diet [10]. At the end of the 4-week run-in period, patients were randomly assigned to 1 of the following 3 groups:

i) One group ($n = 50$) was assigned to a plant stanol ester spread (2 g/day). The spread produced by Minerva Hellas, Greece, contained (per 100 g) 7.1 g plant stanols as ester, 10.5 g saturated fats, <0.5 g trans fats, 20 g monounsaturated fats, 24 g polyunsaturated fats, 800 mg vitamin A, 5 μg vitamin D and 5 mg vitamin E.

ii) The second group ($n = 50$) was assigned to the same spread but without plant stanol esters (placebo) in identical containers as the ''active'' spread.

iii) In the third group ($n = 50$), a structured effort was made to improve adherence to the Mediterranean diet [14–16]. Trained dieticians that provided patients with 7-day menus with food that incorporated the salient characteristics of the Mediterranean diet.

The placebo and the plant stanol ester groups continued with the hypolipidemic diet throughout the intervention period (16 weeks), while the Mediterranean diet group was advised to follow this diet as described above.

At baseline, adherence to the Mediterranean diet over the preceding year was assessed in all groups using a validated semi-quantitative questionnaire [16] that was administered by a trained dietician. A 10-point Mediterranean diet scale was used (range of score, 0–9; higher scores indicate greater compliance). Change in adherence to the Mediterranean diet vs baseline was assessed at the end of the study (after 16 weeks), when patients were asked about their dietary habits during the study period.

3. Main outcome measures: a) Primary endpoint: Change in eCVD risk assessed by the Framingham [http://hp2010.nhlbihin.net/atpiii/riskcalc.htm], the PROspective Cardio- vascular Munster (PROCAM), [http://www.chd-taskforce.com] and the Reynolds [http://www.reynoldsriskscore.org] risk engines at the end of the study vs baseline. b) Secondary endpoints: Change in lipids [TC, LDL-C, high density lipoprotein cholesterol (HDL-C), triglycerides (TGs), apolipoprotein B100 (apo B), apolipoprotein A1 (apo A1)], serum sitosterol and campesterol, blood pressure, haemostatic factors [plasma fibrinogen (F) and plasminogen activator inhibitor (PAI) 1 activity] and inflammatory markers [high sensitivity C-reactive protein (hsCRP)]. c) Tertiary endpoint: Percentage of patients at TC target [<200 mg/dl (5.2 mmol/l)].
4. Exclusion criteria: Patients with established CVD [coronary artery disease (CAD), peripheral arterial disease or symptomatic carotid disease], diabetes mellitus or metabolic syndrome, who would require hypolipidaemic or other drug treatment. Patients with chronic diseases (including liver diseases), pregnancy, malignancies, any drug treatment or unwillingness to participate.
5. Prespecified analyses: a. Postmenopausal women vs women with child bearing potential. b. Patients with impaired fasting glucose [IFG; diabetes was excluded by postprandial glucose concentrations <160 mg/dl (8.9 mmol/l)].

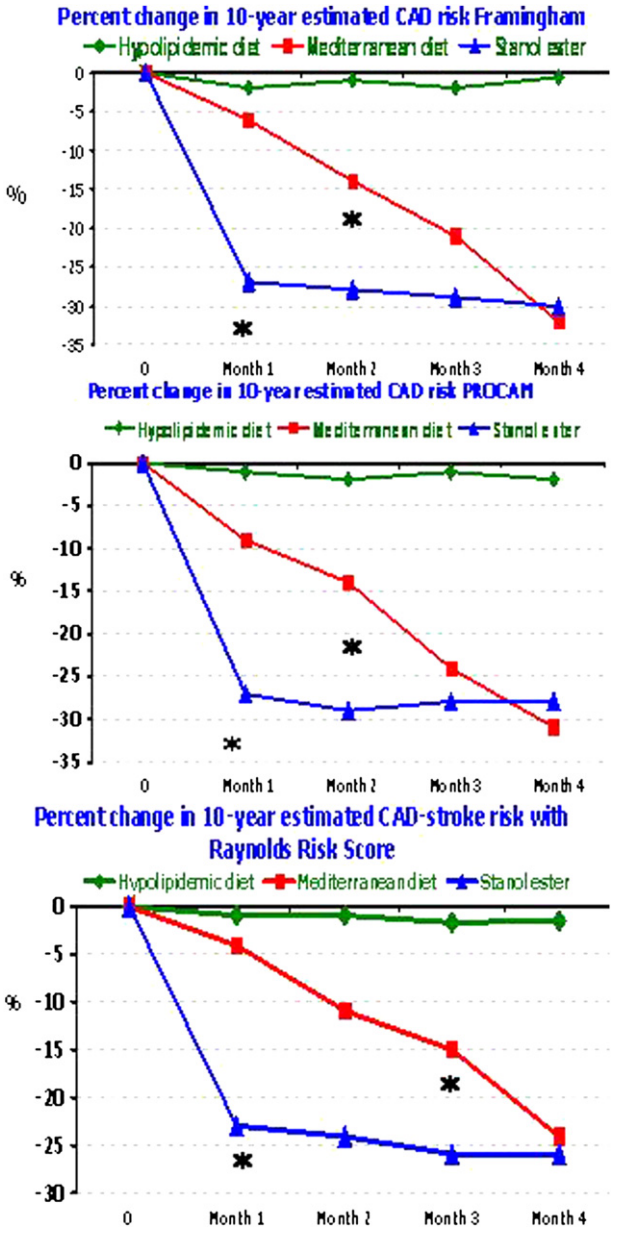


**Figure 2** Time course of change in cardiovascular disease risk in the 3 treatment groups estimated by different risk engines. * = $p < 0.01$ vs placebo spread.

6. Laboratory-based assessments: After an overnight fast of 12 h, TC, HDL-C, TG, apo A1 (reference range 80–110 mg/dl), apo B (reference range 75–115 mg/dl), fasting blood glucose, serum uric acid (SUA), alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase and alkaline phosphatase concentrations/activity were assessed using an Olympus AU 560 autoanalyser and respective reagents (Olympus Diagnostica GmbH, Clare, Ireland). LDL-C was calculated with the Friedewald formula [LDL-C mg/dl = TC in mg/dl-(TG/5 in mg/dl+ HDL-C in mg/dl)]. F was measured by the Clauss method (Fibrindex Ortho Diagnostic, NJ, USA). PAI-1 activity was measured by a chromogenic assay (Spectrolyse-PAI-1, Biopool, Umea, Sweden). hsCRP concentrations were assessed by nephelometry (Alpha Diagnostic

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**Table 1** Patient baseline characteristics.

| Parameter | Control (placebo spread) $n = 50$ | Mediterranean diet $n = 50$ | Plant stanol ester $n = 50$ | $p$ |
|---|---|---|---|---|
| Age (years) | $55.1 \pm 4.1$ | $54.2 \pm 3.5$ | $54.7 \pm 3.7$ | NS |
| Men ($n$) | 24 | 25 | 24 | NS |
| Postmenopausal women ($n$) | 14 | 13 | 13 | NS |
| Smokers (%) | 40 | 39 | 41 | NS |
| Family history of premature CAD (%) | 17 | 18 | 17 | NS |
| Impaired fasting glucose ($n$) | 15 | 14 | 15 | NS |
| Body mass index (kg/m$^2$) | $27.9 \pm 2.1$ | $27.3 \pm 2.5$ | $27.6 \pm 2.2$ | NS |

Values are presented as absolute numbers or as means $\pm$ SD. CAD = coronary artery disease; NS = not significant.

International, San Antonio, Texas, USA-sensitivity 0.35 ng/ml, reference range 0–1 mg/l). Physical examination, measurement of blood pressure, body mass index (BMI), lipid profile, hsCRP, F, PAI-1 activity, fasting blood glucose, serum creatinine, SUA and liver function tests, as well as eCVD risk assessment were performed every month. Serum plant sterols and stanols (sitosterol, sitostanol, campesterol and campestanol) and cholesterol precursors (lathosterol) were measured at baseline and at the end of the study (16th treatment week) by gas chromatography [17]. Samples were stored at $-80\,^{\circ}$C. All samples of 1 subject were analyzed in the same analytical run to exclude variations between runs.

7. Statistical analysis: An intention-to-treat analysis of all patients randomized to the 3 treatment groups was performed. Comparisons between continuous variables were performed using one-way analysis of variance (ANOVA) after adjusting for age and sex. Non-parametric data were compared using the Kruskal–Wallis test. Continuous variables are presented as mean $\pm$ standard deviation. Associations between categorical variables were tested with the Chi-squared test. Differences between continuous variables were tested with the Student's $t$-test and Mann–Whitney test (for normally and non-parametrically distributed variables, respectively). The Bonferroni correction was automatically adjusted to a 2-tailed $p < 0.05$ for multiple comparisons. All analyses were carried out using the SPSS 12.00 software package (SPSS, Inc., Chicago, IL).

## Results

### CVD risk estimates

*Placebo spread group*: there was no change in eCVD.

*Mediterranean diet group*: there was a significant decrease in eCVD risk with all 3 CVD risk engines. The reduction ranged from 24% (Reynolds) to 32% (Framingham) (Fig. 2).

*Plant stanol ester group*: the decrease in TC and LDL-C concentrations were the main contributors to the significant reduction in eCVD risk according to all 3 risk engines. These reductions [ranging from 26%-Reynolds to 30%-Framingham (Fig. 2)] were manifested as early as the 1st treatment month and were equaled by eCVD risk reductions of the Mediterranean diet group only at the 4$^{th}$ treatment month.

Both interventions (Mediterranean and plant stanol ester) had similar effects on eCVD risk factors and eCVD risk in postmenopausal women and in patients with IFG as in women of child bearing potential and patients with normal glucose concentrations, respectively.

### Adherence to the Mediterranean diet-changes in serum lipids-lipoproteins and other CVD risk factors

After the 4-week run-in period (lifestyle advice only), all subjects continued to fulfill the inclusion criteria [TC 200–250 mg/dl (5.2–6.4 mmol/l)] and entered the intervention period. Values obtained at this visit were considered as baseline data (Tables 1 and 2).

Patients in the placebo spread group had no significant change in either CVD risk factors or diet score (Tables 2 and 3; Fig. 2).

Adherence to the Mediterranean diet score during the preceding year was approximately 3 in all 3 groups using the 10-point scale [14–16]. In the placebo spread and plant stanol ester groups this score did not change significantly during the study. In contrast, in the Mediterranean diet group, there was a significant improvement in adherence to the Mediterranean diet [a 2.2-points mean increase (from 3.2 to 5.4, $p < 0.001$)]. This was associated with a gradual reduction in TC, LDL-C, TGs, systolic and diastolic blood pressure, hsCRP, F and PAI-1 activity, as well as with an increase in HDL-C concentrations (Table 2, Fig. 3a and b).

In the plant stanol ester group there was a significant decrease in TC (−14%) and LDL-C (−16%) concentrations, recorded at the 1st intervention month. TC and LDL-C concentrations remained steady throughout the study. (Fig. 3a). CRP was also significantly reduced (by 17%, $p = 0.002$). (Table 2, Fig. 3b). LDL-C reduction preceded the fall in hsCRP and was mainly responsible for the eCVD risk reduction. TGs, HDL-C, blood pressure, F and PAI-1 activity improved, but not significantly (Table 2).

Liver and kidney function tests, SUA concentrations, BMI, and smoking status remained unaffected during the 16-week duration of the study in all groups (data not shown).

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**Table 2** Effects of placebo, Mediterranean diet and plant stanol esters on cardiovascular risk factors during the study.

| | Control (placebo ester) $n = 50$ | Mediterranean diet $n = 50$ | Plant stanol ester $n = 50$ |
|---|---|---|---|
| Total cholesterol (mg/dl) | | | |
| Baseline | 223 ± 14 | 225 ± 12 | 228 ± 15 |
| End of study | 219 ± 13 | 208 ± 13 | 193 ± 10 |
| Change % | −1.8 | −7**† | −14**††‡ |
| Triglycerides (mg/dl) | | | |
| Baseline | 115 ± 26 | 113 ± 23 | 112 ± 22 |
| End of study | 114 ± 25 | 106 ± 20 | 110 ± 21 |
| Change % | −1 | −6**†& | −2 |
| High density lipoprotein cholesterol (mg/dl) | | | |
| Baseline | 45 ± 6 | 45 ± 6 | 45 ± 7 |
| End of study | 45 ± 6 | 48 ± 6 | 46 ± 6 |
| Change % | — | 6%*†& | 2 |
| Low density lipoprotein cholesterol (mg/dl) | | | |
| Baseline | 156 ± 17 | 158 ± 16 | 159 ± 19 |
| End of study | 152 ± 15 | 143 ± 16 | 133 ± 16 |
| Change % | −2 | −9*† | −16**††‡ |
| Systolic blood pressure (mmHg) | | | |
| Baseline | 124 ± 9 | 123 ± 8 | 122 ± 8 |
| End of study | 122 ± 8 | 118 ± 7 | 120 ± 9 |
| Change % | −2 | −4*† | −2 |
| Diastolic blood pressure (mmHg) | | | |
| Baseline | 75 ± 5 | 76 ± 6 | 75 ± 7 |
| End of study | 74 ± 5 | 74 ± 5 | 74 ± 6 |
| Change % | −1 | −3 | −1 |
| Plasma glucose (mg/dl) | | | |
| Baseline | 89 ± 11 | 91 ± 9 | 92 ± 10 |
| End of study | 87 ± 10 | 86 ± 7 | 89 ± 8 |
| Change % | −2 | −6*† | −3 |
| High sensitivity C-reactive protein (mg/l) | | | |
| Baseline | 2.3 ± 0.6 | 2.2 ± 0.6 | 2.1 ± 0.6 |
| End of study | 2.3 ± 0.6 | 1.8 ± 0.5 | 1.8 ± 0.5 |
| Change % | — | −19**†† | −17**†† |
| Fibrinogen (mg/l) | | | |
| Baseline | 3.02 ± 0.23 | 2.92 ± 0.19 | 2.97 ± 0.25 |
| End of study | 2.96 ± 0.22 | 2.66 ± 0.16 | 2.90 ± 0.23 |
| Change % | −2 | −9**†& | −2 |
| Plasminogen activator inhibitor type 1 activity (AU/dl) | | | |
| Baseline | 10.9 ± 3.7 | 11.7 ± 1.7 | 11.2 ± 4.4 |
| End of study | 11.2 ± 3.7 | 10.4 ± 1.5 | 10.9 ± 4.2 |
| Change % | 3 | −11**††& | −3 |

*$p < 0.01$ vs baseline, **$p < 0.002$ vs baseline, †$p < 0.01$ vs control, ††$p < 0.001$ vs control, ‡$p < 0.003$ vs Mediterranean diet, & $p < 0.01$ vs plant stanol ester.

## Serum concentrations of plant sterols/stanols and cholesterol precursors

Compared with the control and the Mediterranean diet groups, cholesterol-standardized serum plant sterol concentrations (sitosterol and campesterol) significantly decreased (−52% and −32%, respectively; $p < 0.001$ for both changes) in the plant stanol ester group, indicating a reduction in cholesterol absorption (Table 3). The cholesterol-standardized concentration of the cholesterol precursor lathosterol increased significantly (by 21%; $p < 0.001$) in the plant stanol ester group compared with non-significant reductions in the control and Mediterranean diet group (by 1 and 2%, respectively), indicating an (probably compensatory) increase in cholesterol synthesis (Table 3). The net result of the reduction in cholesterol

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**Table 3** Effects of placebo, Mediterranean diet and plant stanol esters use on cholesterol-standardized serum plant sterol and stanol levels during the study.

| | Control (placebo ester) $n = 50$ | Mediterranean diet $n = 50$ | Plant stanol ester $n = 50$ |
|---|---|---|---|
| Serum sitosterol ($10^2 \times \mu$mol/mmol cholesterol)(multiplication) | | | |
| Baseline | $85.1 \pm 15.4$ | $84.0 \pm 16.5$ | $87.2 \pm 15.7$ |
| End of study | $84.6 \pm 16.1$ | $86.6 \pm 16.5$ | $43.2 \pm 9.4$ |
| Change % | −2% | 2% | −52%*†‡ |
| Serum campesterol ($10^2 \times \mu$mol/mmol cholesterol) | | | |
| Baseline | $187.9 \pm 43.4$ | $188.9 \pm 44.1$ | $190.3 \pm 45.2$ |
| End of study | $185.6 \pm 42.36$ | $194.7 \pm 46.6$ | $110.6 \pm 31.2$ |
| Change % | −1% | 3% | −32%*†‡ |
| Serum lathosterol ($10^2 \times \mu$mol/mmol cholesterol) | | | |
| Baseline | $78.4 \pm 15.2$ | $77.5 \pm 14.5$ | $78.2 \pm 14.9$ |
| End of study | $77.6 \pm 15.3$ | $75.5 \pm 13.8$ | $94.3 \pm 16.5$ |
| Change % | −1% | −2% | 21%*†‡ |
| Serum sitostanol ($10^2 \times \mu$mol/mmol cholesterol) | | | |
| Baseline | $7.7 \pm 4.2$ | $7.5 \pm 4.3$ | $7.9 \pm 4.5$ |
| End of study | $7.6 \pm 4.2$ | $7.8 \pm 4.4$ | $12.3 \pm 5.7$ |
| Change % | −1 | 4 | 56*†‡ |
| Serum campestanol ($10^2 \times \mu$mol/mmol cholesterol) | | | |
| Baseline | $8.3 \pm 4.9$ | $8.6 \pm 4.6$ | $8.5 \pm 4.8$ |
| End of study | $8.4 \pm 4.9$ | $9.0 \pm 4.7$ | $12.1 \pm 5.9$ |
| Change % | 1 | 5 | 42*†‡ |

*$p < 0.0001$ vs baseline, †$p < 0.0001$ vs control, ‡$p < 0.0001$ vs Mediterranean diet.

absorption and the increase in cholesterol synthesis with plant stanol esters was a significant reduction in serum cholesterol concentrations. Cholesterol-standardized sitostanol and campestanol concentrations significantly increased in the plant stanol ester group (Table 3). This suggests that plant stanols are absorbed to a small extent. However, cholesterol-standardized serum levels of plant stanols remained very low (Table 3).

### Percentage of subjects at lipid target

At the end of the study, 7/50, 23/50 and 40/50 patients in the control (placebo), Mediterranean diet and plant stanol ester groups achieved TC levels <200 mg/dl (5.2 mmol/l; $p = 0.0001$ plant stanol ester spread vs placebo spread, $p = 0.0008$ for plant stanol ester spread vs Mediterranean diet and $p = 0.0009$ for placebo spread vs Mediterranean diet).

## Discussion

An increased adherence to the Mediterranean diet (by 2.2 points on a 10-point scale) in mildly hypercholesterolaemic subjects was associated with a substantial reduction in eCVD risk due to changes in several CVD risk factors. Plant stanol ester use was also associated with a significant (and similar to the Mediterranean diet) decrease in eCVD risk, compared with placebo, mainly due to a steady reduction in LDL-C concentrations. Therefore, stanol ester consumption appears to be a useful option to reduce CVD risk in moderately hypercholesterolaemic subjects who do not adopt a Mediterranean diet.

Several studies showed the beneficial role of the Mediterranean diet (rich in fruits, vegetables, olive oil, legumes, whole grains, fish, nuts and low-fat dairy products) on risk of CVD, T2DM and several types of cancer [14–16,18–20]. Prospective data showed that a 2-point increase in the 10-point scale we used corresponds to a 25% reduction in total mortality [15,16]. The Mediterranean diet is also associated with beneficial effects on blood pressure, lipoproteins, platelet aggregation, inflammation and coagulation [19,21,22]. Our results are in agreement with those mentioned above. We found that improved adherence to the Mediterranean diet was associated with beneficial changes in TC, LDL-C, TGs and HDL-C concentrations and reductions in systolic blood pressure, inflammatory (hsCRP) and thrombotic markers (F concentrations and PAI-1 activity). It therefore appears that the Mediterranean diet reduces vascular risk through several mechanisms. We observed an approximately 30% reduction in eCVD risk associated with a 2.2-point increase in this 10-point scale in agreement with previous studies [15,16]. The changes in CVD risk factors and eCVD risk occurred in a population with a baseline adherence to Mediterranean diet of 3.2 points. If mildly hypercholesterolaemic subjects from countries with lower baseline scores could attain such a high level of adherence (5.4 points) the benefit could be more evident.

Several reports showed a favourable impact of food products with added sterol or stanol on TC and LDL-C concentrations in subjects with either normal or elevated TC concentrations [23–28]. These studies indicate that adding 2–3 g/day of plant stanols to an ''ordinary'' or low-fat diet lowers LDL-C concentrations by 15% or more

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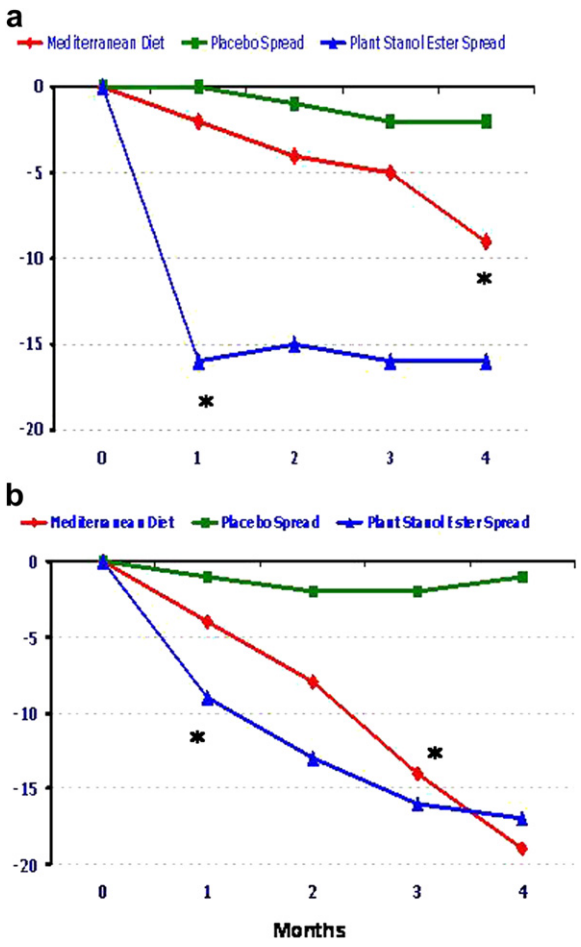


**Figure 3** Time course of change in LDL-C and hsCRP in the 3 treatment groups. * = $p < 0.01$ vs placebo spread.

[23—28]. Our findings in a Mediterranean population are in agreement with those of the above studies that included North European or US populations. This was the reason for this patient group reaching cholesterol targets more than the other 2 groups in our study. A reduction in LDL-C concentrations of this magnitude would be expected to reduce the risk of CAD by about 25% [28], similar to that achieved by a 2-point increase in the 10-point Mediterranean diet scale mentioned above.

The hsCRP decrease in the Mediterranean diet group might play a role in eCVD risk reduction. However, only the Reynolds risk engine includes hsCRP. It was shown that adherence to the Mediterranean diet attenuates pro-inflammatory processes in healthy adults [22]. In addition, elevated hsCRP concentrations are independently associated with increased CVD risk [29]. Increased adherence to Mediterranean diet was related to lower hsCRP concentrations and reduced CVD risk [29]. Previous studies reported that plant stanol ester consumption, on top of statin treatment, reduced hsCRP concentration by 37% [30], a reduction greater than what we observed (−17%). However, in another study using both plant sterols and stanols in 41 subjects on statins there was no change in hsCRP concentration [31]. It appears that the elevated hsCRP baseline concentrations, despite statin treatment, might have played a role in the significant reduction in the first study [30]. Mean baseline hsCRP concentrations were approximately 2 mg/l in our patients probably because of the presence of a combination of CVD risk factors [32,33]. All patients had dyslipidaemia, a substantial number were smokers and some had positive family history of premature CAD or IFG (Table 1). Our patients share some similarities with those enrolled in the primary prevention trial ''Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin'' (JUPITER) [33]. It was shown that statin therapy reduces hsCRP concentrations. The magnitude of clinical benefit associated with statin therapy correlates in part with the achieved hsCRP level [34]. The JUPITER trial [33] showed that patients without established CVD, like those included in our study, with hsCRP concentrations >2 mg/l, regardless of their LDL-C concentration, have an increased risk for CVD. This risk was substantially reduced (by 44% vs placebo) with rosuvastatin [33]. Thus, achieving LDL-C targets and hsCRP concentrations <2 mg/L was associated with improved event-free survival compared with achieving neither target or LDL-C targets alone [33,35,36]. This is also probably the case in our study, where reductions in both LDL-C and hsCRP (to a lesser degree than in those in JUPITER) were recorded in both active dietary intervention groups. We observed a beneficial effect of Mediterranean diet on F concentrations and PAI-1 activity, in accordance with previous studies [22], while in the placebo and plant stanol ester groups there was no significant change in these parameters [37].

In the plant stanol group, we observed a substantial reduction ($p < 0.001$) compared with baseline and the other 2 groups in serum plant sterol (principally sitosterol) concentrations (indicating a reduction in cholesterol absorption). In patients with sitosterolaemia, mutations in the ABCG5 or ABCG8 genes result in a marked increase in serum plant sterol concentrations, which are associated with premature CAD [38], similar to patients with homozygous familial hypercholesterolaemia [39]. Some data suggest that there is no association between moderately elevated serum concentrations of plant sterols and atherosclerosis in either animals or humans [40—42]. In contrast, a study in patients with a positive family history for CAD showed that moderate but significant elevations in values of serum sitosterol and campesterol were independently associated with increased CAD risk [43]. In a nested case-control *post hoc* analysis of the PROCAM trial, moderately elevated sitosterol concentrations (>5.25 μmol/l) were also associated with an approximately 3-fold increase in the risk of a CAD event in high risk men, during a 10-year follow-up [44]. This was also shown in post-menopausal CAD women during a 5-year follow-up [45]. In Finnish patients of the Scandinavian Simvastatin Survival Study (4S) with high (baseline and during study) serum plant sterol concentrations there was no benefit (reduction in CAD recurrence) during the 5-year treatment with simvastatin [46]. The 4S investigators suggested additional treatment with inhibitors of cholesterol and plant sterol absorption (e.g. with plant stanol esters) for this patient group [47]. Moreover, higher serum plant sterol concentrations are associated with higher concentrations in atherosclerotic plaques [48]. Use of ezetimibe (an intestinal cholesterol and plant sterol absorption inhibitor) results in a marked reduction in serum sitosterol and campesterol

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concentrations, regression of xanthomatosis, resolution of carotid bruits and improvement in aortic stenosis in patients with sitosterolaemia [49,50]. These findings suggest that plant stanol supplements might exert an additional benefit by lowering circulating plant sterol concentrations.

## Conclusions

Our findings suggest that the Mediterranean diet has beneficial effects on several vascular risk factors and reduces eCVD risk in patients with mild hypercholesterolaemia. Plant stanol esters conferred a similar reduction in eCVD risk resulting mainly from the decrease in TC and LDL-C concentrations. This was evident among mildly hypercholesterolaemic men or women, postmenopausal women and patients with IFG. It appears that the use of plant stanol esters by moderately hypercholesterolaemic subjects is a useful option to reduce CVD risk in those who do not adopt a Mediterranean diet.

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## References

[1] Martínez-González MA, de la Fuente-Arrillaga C, Nunez-Cordoba JM, Basterra-Gortari FJ, Beunza JJ, Vazquez Z, et al. Adherence to Mediterranean diet and risk of developing diabetes: prospective cohort study. BMJ 2008;14(336):1348—51.
[2] Trichopoulou A, Critselis C. Mediterranean diet and longevity. Eur J Cancer Prev 2004;13:453—6.
[3] Knoops KT, de Groot LC, Kromhout D, Perrin AE, Moreiras-Varela O, Menotti A, et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. J Am Med Assoc 2004;292:1433—9.
[4] Willet WC. The Mediterranean diet: science and practice. Public Health Nutr 2006;9:105—10.
[5] Mitrou PN, Kipnis V, Thiébaut AC, Reedy J, Subar AF, Wirfält E, et al. Mediterranean dietary pattern and prediction of all-cause mortality in a US population: results from the NIH-AARP Diet and Health Study. Arch Intern Med 2007;167:2461—8.
[6] Rodrigues SS, Caraher M, Trichopoulou A, de Almeida MD. Portuguese households' diet quality (adherence to Mediterranean food pattern and compliance with WHO population dietary goals): trends, regional disparities and socioeconomic determinants. Eur J Clin Nutr 2008;62:1263—72.
[7] Sofi F, Innocenti G, Dini C, Masi L, Battistini NC, Brandi ML, et al. Low adherence of a clinically healthy Italian population to nutritional recommendations for primary prevention of chronic diseases. Nutr Metab Cardiovasc Dis 2006;16:436—44.
[8] Correa Leite ML, Nicolosi A, Cristina S, Hauser WA, Pugliese P, Nappi G. Dietary and nutritional patterns in an elderly rural population in Northern and Southern Italy: (I). A cluster analysis of food consumption. Eur J Clin Nutr 2003;57:1514—21.
[9] Sofi F, Vecchio S, Giuliani G, Martinelli F, Marcucci R, Gori AM, et al. Dietary habits, lifestyle and cardiovascular risk factors in a clinically healthy Italian population: the ''Florence'' diet is not Mediterranean. Eur J Clin Nutr 2005;58:584—91.
[10] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). J Am Med Assoc 2001;285:2486—97.
[11] Grundy SM. Stanol esters as a component of maximal dietary therapy in the national cholesterol education program adult treatment Panel III Report. Am J Cardiol 2005;96(Suppl. 1):47—50.
[12] Plat J, Mensink RP. Increased intestinal ABC A1 expression contributes to the decrease in cholesterol absorption after plant stanol consumption. FASEB J 2002;16:1248—53.
[13] Escurriol V, Cofán M, Serra M, Bulló M, Basora J, Salas-Salvadó J, et al. Serum sterol responses to increasing plant sterol intake from natural foods in the Mediterranean diet. Eur J Nutr 2009 May 3 [Epub ahead of print].
[14] Trichopoulou A, Kouris-Blazos A, Wahlqvist ML, Gnardellis C, Lagiou P, Polychronopulos E, et al. Diet and overall survival in the elderly. BMJ 1995;311:1457—60.
[15] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599—608.
[16] Panagiotakos DB, Pitsavos C, Arvaniti F, Stefanadis C. Adherence to the Mediterranean food pattern predicts the prevalence of hypertension, hypercholesterolemia, diabetes and obesity, among healthy adults; the accuracy of the MedDietScore. Prev Med 2007;44:335—40.
[17] Plat J, Mensink RP. Effects of diets enriched with two different plant stanol ester mixtures on plasma Ubiquinol-10 and fat-soluble antioxidant concentrations. Metabolism 2001;50:520—9.
[18] Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, et al. The diet and 15-year death rate in the Seven Countries Study. Am J Epidemiol 1986;124:903—15.
[19] De Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. Circulation 1999;99:779—85.
[20] Kafatos A, Diacatou A, Voukiklaris G, Nikolakakis N, Vlachonikolis J, Kounali D, et al. Heart disease risk-factor status and dietary changes in the Cretan population over the past 30 years: the Seven Countries Study. Am J Clin Nutr 1997;65:1882—6.
[21] Barradas MA, Christofides JA, Jeremy JY, Mikhailidis DP, Fry DE, Dandona P. The effect of olive oil supplementation on human platelet function, serum cholesterol-related variables and plasma fibrinogen concentrations: a pilot study. Nutr Res 1990;10:403—11.
[22] Chrysohoou C, Panagiotakos DB, Pitsavos C, Das UN, Stefanadis C. Adherence to the Mediterranean diet attenuates inflammation and coagulation process in healthy adults. The Attica study. J Am Coll Cardiol 2004;44:152—8.
[23] Weststrate JA, Meijer GW. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterorolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1998;52:334—43.
[24] Nguyen TT, Dale LC, von Bergmann K, Croghan IT. Cholesterol-lowering effect of stanol ester in a US population of mildly hypercholesterolemic men and women: a randomized controlled trial. Mayo Clin Proc 1999;74:1198—206.
[25] Hendriks HF, Weststrate JA, van Vliet T, Meijer GW. Spreads enriched with three different levels of vegetable oil sterols and the degree of cholesterol lowering in normocholesterolaemic and mildly hypercholesterolaemic subjects. Eur J Clin Nutr 1999;53:319—27.

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[26] Hallikainen MA, Sarkkinen ES, Gylling H, Erkkilä AT, Uusitupa MI. Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet. Eur J Clin Nutr 2000;54:715–25.
[27] Plat J, van Onselen EN, van Heugten MM, Mensink RP. Effects on serum lipids, lipoproteins and fat soluble antioxidant concentrations of consumption frequency of margarines and shortenings enriched with plant stanol esters. Eur J Clin Nutr 2000;54:671–7.
[28] Law MR. Plant sterol and stanol margarines and health. BMJ 2000;320:861–4.
[29] Panagiotakos DB, Pitsavos C, Chrysohoou C, Skoumas I, Stefanadis C. ATTICA Study. Five-year incidence of cardiovascular disease and its predictors in Greece: the ATTICA study. Vasc Med 2008;13:113–21.
[30] Cater NB, Garcia-Garcia AB, Vega GL, Grundy SM. Responsiveness of plasma lipids and lipoproteins to plant stanol esters. Am J Cardiol 2005;96(1A):23D–8D.
[31] De Jong A, Plat J, Bast A, Godschalk RW, Basu S, Mensink RP. Effects of plant sterol and stanol ester consumption on lipid metabolism, antioxidant status and markers of oxidative stress, endothelial function and low-grade inflammation in patients on current statin treatment. Eur J Clin Nutr 2008; 62:263–73.
[32] Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959–65.
[33] Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr AM, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. N Engl J Med 2008;359:2195–207.
[34] Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
[35] Tziomalos K, Athyros VG, Karagiannis A, Mikhailidis DP. JUPITER: major implications for vascular risk assessment. Curr Med Res Opin 2009;25:133–7.
[36] Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr AM, Kastelein JJ, et al. Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial. Lancet 2009;373:1175–82.
[37] Plat J, Mensink RP. Vegetable oil based versus wood based stanol ester mixtures: effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. Atherosclerosis 2000;148:101–12.
[38] Salen G, Shefer S, Nguyen L, Ness GC, Tint GS, Shore V. Sitosterolemia. J Lipid Res 1992;33:945–55.
[39] Glueck CJ, Speirs J, Tracy T, Streicher P, Illig E, Vandegrift J. Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hyperphytosterolemic probands and their first-degree relatives. Metabolism 1991;40:842–8.
[40] Wilund KR, Yu L, Xu F, Vega GL, Grundy SM, Cohen JC, et al. No association between plasma levels of plant sterols and atherosclerosis in mice and men. Arterioscler Thromb Vasc Biol 2004;24:2326–32.
[41] Plat J, Beugels I, Gijbels MJJ, de Winther MPJ, Mensink RP. Plant sterol or stanol esters retard lesion formation in LDL receptor-deficient mice independent of changes in serum plant sterols. J Lipid Res 2006;47:2762–71.
[42] Fassbender K, Lütjohann D, Dik MG, Bremmer M, König J, Walter S, et al. Moderately elevated plant sterol levels are associated with reduced cardiovascular risk-The LASA study. Atherosclerosis 2008;196:283–8.
[43] Sudhop T, Gottwald BM, von Bergmann K. Serum plant sterols as a potential risk factor for coronary heart disease. Metabolism 2002;51:1519–21.
[44] Assmann G, Paul Cullen P, Erbey J, Rameyd DR, Kannenberga F, Schulte H. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: results of a nested case-control analysis of the Prospective Cardiovascular Münster (PROCAM) study. Nutr Metab Cardiovasc Dis 2006;16:13–21.
[45] Rajaratnam RA, Gylling H, Miettinen TA. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. J Am Coll Cardiol 2000;35:1185–91.
[46] Miettinen TA, Gylling H, Strandberg T, Sarna S, for the Finnish 4S Investigators. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian Simvastatin Survival Study (4S). Br Med J 1998;316:1127–30.
[47] Miettinen TA, Strandberg TE, Gylling H, for the Finnish Investigators of the Scandinavian Simvastatin Survival Study Group. Non-cholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients: relation to basal serum cholestanol. Arterioscler Thromb Vasc Biol 2000;20:1340–6.
[48] Miettinen TA, Railo M, Lepäntalo M, Gylling H. Plant sterols in serum and in atherosclerotic plaques of patients undergoing carotid endarterectomy. J Am Coll Cardiol 2005;45: 1794–801.
[49] Salen G, von Bergmann K, Lütjohann D, Kwiterovich P, Kane J, Patel SB, et al, the Multicenter Sitosterolemia Study Group. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. Circulation 2004;109:966–71.
[50] Salen G, Starc T, Sisk CM, Patel SB. Intestinal cholesterol absorption inhibitor ezetimibe added to cholestyramine for sitosterolemia and xanthomatosis. Gastroenterology 2006; 130:1853–7.

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