# Reduction of LDL-Cholesterol in Mildly Hypercholesterolemic Thais with Plant Stanol Ester-Fortified Soy Milk

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***Objective:*** *To investigate the effects of soymilk fortified with plant stanol ester on changes in LDL-cholesterol, fat-soluble vitamins and sex hormones in Thai volunteers.*
***Material and Method:*** *In a double-blind, placebo-controlled study, 120 mildly hypercholesterolemic Thais were randomly assigned to stanol and control groups that were comparable in lipid profile and body mass index. Subjects consumed regular or 2g stanol-containing soymilk once a day and postprandially for six weeks. The serum lipid profile was measured at weeks 0, 2 and 6; serum fat-soluble vitamins and sex hormones were measured at weeks 0 and 6.*
***Results:*** *The mean reduction in total cholesterol was 8.2 % in the stanol group ($p < 0.0001$) and 0.6% in the control group. LDL-cholesterol declined in both groups at week two, but the reduction was maintained to week six only in the stanol group. The mean reduction in LDL-cholesterol was 13.5% in the stanol group ($p < 0.0001$) at week 6, compared to a 4.6% decrease in the control group. Adjusted serum β-cryptoxantene and β-carotene levels decreased at week six for the stanol group. Serum sex hormone levels in both groups remained unchanged.*
***Conclusion:*** *Consumption of stanol-ester-containing soymilk for six weeks significantly reduced LDL-cholesterol in mildly hypercholesterolemic Thais. No adverse effect on sex hormones was observed. However, stanol-ester consumers are at risk of fat-soluble-vitamin deficiencies if the vitamin intake from foods is inadequate.*

***Keywords:*** *Plant stanol esters, Soy milk, LDL-cholesterol, Mildly hypercholesterolemic, Thai*

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National Cholesterol Education Program guidelines[1] and the American Heart Association[2] recommend phytosterols and their esters as a cholesterol-lowering therapeutic option, in addition to diet and lifestyle modification, for lowering coronary heart disease risk. They act synergistically with dietary and pharmacologic treatments, compare favorably with medications in overall cost and safety, and provide patients with the chance to discontinue the drugs or take them at a lower dose[3]. Structurally related to cholesterol, but hardly absorbed by the body, their presence in the intestine interferes with the absorption of exogenous and endogenous cholesterol[4,5], with a resultant drop in serum cholesterol levels mainly affecting total cholesterol (TC) and LDL-cholesterol (LDL-c) concentrations[4,6-8].

The review of randomized placebo-controlled trials by Katan M.B et al [9] indicated that 2-3 g plant sterols/stanols daily was the cost-effective dose for lowering LDL-c by 10-13% in individuals with normal or increased levels of cholesterol. This amount is unlikely to be sufficiently provided through such natural sources as rapeseed oil, tall oil, soybean oil, cereals, legumes, nuts, and other plants[10, 11]. Average daily plant sterols intake in typical Western diets varies from 150-400 mg/day[9]. Plant stanols, the saturated counterparts of plant sterols, occur more scarcely in nature, and are estimated at only 20-50 mg/d in average Western diets[12].

Because phytosterols prevent cholesterol absorption, their affect can impact fat-soluble vitamins. Decreased plasma levels of α-carotene, β-carotene, α-tocopherol, and lycopene were reported when phytosterols were taken[9,13-16]. However, after LDL-c

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standardized, concentrations of the lipid-soluble antioxidants did not change significantly in some studies[17,18]. The hypothetical effect of reduced cholesterol absorption on serum sex hormones has also been proposed, even though such adverse effects have so far been demonstrated only from parenteral administration of large doses in experimental animals[19].

Phytosterol fortified foods such as margarine, spread, milk-type products, and baked goods, which, although available in European and American markets, do not yet constitute a major part of most Asian diets. Alternatively, due to the popularity of soymilk among Asians, soymilk enriched with plant stanol ester is being pursued in Thailand. Since the background diet of individuals may influence the lipid-lowering effects of plant stanol ester[20], a need exists to investigate: (i) whether plant stanol carried by such a non-fatty food product as soy milk will result in similar beneficial outcomes among the Thai population, (ii) whether their consumption in the background of habitual eating pattern will result in the decrease of fat-soluble vitamins, and (iii) whether the sex hormone balance will accompany reduced absorption of cholesterol.

## Material and Method

### *Subjects*

All volunteers were recruited via Intranet and posters at the Salaya Campus of Mahidol University, Thailand. Mahidol University employees and individuals from the surrounding area (146), who were willing to participate in the present study, were invited for interview and blood screening. The primary selection criteria were: ages between 25 and 60 years; serum TC concentration $\geq$ 5.20 mmol/L; serum LDL-c concentration $\geq$ 3.36 mmol/L; serum triglyceride concentration $<$ 5.10 mmol/L; absence of renal, diabetes, hepatic thyroid or alcohol problems; and pre-test willingness to consume the test or control product throughout the present study's six-week duration. Exclusion criteria included the use of cholesterol-lowering medication, plant stanol, plant sterol products or any supplements; body mass index $>$ 35 kg/m$^2$; pregnancy or lactation; alcohol or drug addiction; insulin-treated diabetes; or instable coronary artery disease. Based on these criteria, 120 volunteers were accepted as participants in the present study. The sample size was determined to be 50 persons for each group based on Miettinen TA's study[7] of a mean reduction of 10% of LDL cholesterol with plant sterols with $\alpha$ error at the 5% level and $\beta$ error 0.20. Another 20% drop-out was added; therefore, approximately 60 volunteers per group were recruited. The Ethics Committee of Mahidol University Institutional Review Board approved the study's design. Written informed consent was obtained from the participants before starting the present study.

### *Study design*

In this randomized, double-blind, placebo-controlled study, stanol-ester-containing strawberry-flavored soymilk (stanol group) was compared with strawberry-flavored soymilk (control group). Table 1 gives the composition of both products. After stratification by gender and levels of LDL cholesterol, 120 volunteers were randomized to stanol and control groups. They were instructed to drink the soy beverage once a day within 30 minutes after lunch or dinner for six weeks while maintaining their habitual diet, physical activity and other lifestyle. The placebo and experimental soymilks were similar in color, taste, fat content, and caloric value to preserve the study's double-blind design; a three-digit numerical code was assigned to each product. The codes were revealed after all analyses were performed. The beverages were distributed every two weeks, at which time compliance and side effects were checked.

### *Dietary record*

Volunteers were asked to record the type and amount of all foods consumed on Thursday,

**Table 1.** Ingredient and nutrient composition for control and stanol-ester-containing soy milk

| Composition | Control soy milk | Stanol-ester soy milk |
|---|---|---|
| Ingredient | | |
| Water (%) | 77.04 | 77.32 |
| Strawberry juice (%) | 15.00 | 15.00 |
| Isolated soy protein (%) | 1.27 | 1.23 |
| Xylitol (%) | 1.56 | 1.50 |
| Aspartame and Acesilfame K (%) | 0.02 | 0.02 |
| Coffee creamer (%) | 5.11 | 0 |
| Plant stanol ester (%) | 0 | 4.86 |
| Nutrient (per 1 bottle, 70 mL) | | |
| Energy (kcal) | 41.6 | 40.00 |
| Total carbohydrate (g) | 6.22 | 2.29 |
| Sugar (g) | 1.98 | 1.69 |
| Dietary fiber (g) | 0.66 | 0.41 |
| Protein (g) | 1.00 | 0.80 |
| Fat (exclude plant stanols) (g) | 1.41 | 0.98 |
| Total plant stanol (g) | 0 | 2.00 |

Friday, and Saturday or Sunday before the present study began and prior to the biweekly schedules. Dietitians trained volunteers to use scales, measuring spoons, and cups to estimate portion sizes. At each submission, dietary records were reviewed to resolve any uncertainty in the entries and to assess record completeness. The completed diet records were evaluated for energy, carbohydrate, protein, and fat intakes, using the INMUCAL software (version WD.1.1, Institute of Nutrition, Mahidol University, Thailand).

***Anthropometric measurements***

The height of each subject was measured using a stadiometer to the nearest 0.1 cm. Body weight was measured using a digital weighing scale (Soehnle 7755, Germany) while the subject was standing without shoes and dressed in indoor clothing. Body weight and height were measured at the beginning and at the end of week 6 of the present study. Body-mass index (BMI) was calculated as weight in kg/height squared in meter.

***Biochemical indicators***

12-hour-fast blood samples were taken before the present study began, at the end of week two, and at the end of the study. All samples from the beginning and the end of the present study were measured for serum TC, LDL-c, HDL-cholesterol (HDL-c), and triglyceride. Concentrations of lipid parameters were analyzed by enzymatic colorimetric methods using the Olympus/AU 400 auto analyzer. Interassay variability was 2.1% for TC, 3.1% for LDL-c, 2.0% for HDL-c, and 2.9% for triglycerides. Serum retinol, β-carotene, and α-tocopherol levels were measured using high-pressure liquid chromatography (HPLC). Serum samples contained all internal standards, precipitated protein by ethanol, and then extracted with hexane. The Waters Alliance HPLC System included a Supelcosil™ LC-18 (5 µm, 250 x 4.6 mm, USA) and compatible guard column C-18 (Supelguard 2 cm L x 2.1 mm ID), 60 µl Autoinjector (Waters 717), and PDA (Waters 996) detector: VIS at 450 nm for carotenoids, UV at 325 nm for retinol, and UV at 295 nm for tocopherol. The chromatography mobile phase was a mixture of $CH_3CN$/ THF/ $CH_3OH$/ TEA (80:14:6:0.1) containing 0.2% Ammonium acetate with flow isocratic 1.5 ml/min (Waters 515). Areas of all chromatograms were measured by Millenium software from Waters, and calculated amount of retinol, tocopherol, and carotenoid (mmol/L) by internal standard method. Serum testosterone and estradiol levels were measured from randomly selected blood samples taken at the beginning and end of the present study from nine male volunteers and 20 female volunteers, respectively, per group. Blood samples from all 29 volunteers were used for the analysis of serum luteinizing hormones (LH). Levels of estradiol and LH were performed with a microparticle enzyme immunoassay technique (IMx, Abbott Laboratories, Abbott Park, Ill; the intraassay coefficients of variation were 6.1 and 6.4 for estradiol and LH, respectively). Serum testosterone levels were measured using an automated direct chemiluminescent immunoassay (Advia Centaur System, Bayer Diagnostics, Newbury, UK; the intraassay coefficient of variation was 4.7%).

***Statistical analysis***

Normality and homogeneity of variance assumptions were checked before further analysis using the Kolgororov-Sminov Test. Student's t test was used for the comparison of dietary intake between groups. Analysis of variance for repeated measurements was used for the hypothesis testing of effect of stanol ester on lipid profile. Changes for all parameters were calculated for each subject as the difference between zero-week and two-week or zero-week and six-week. The differences in changes between the groups were tested with an unpaired t-test or Mann-Whitney U test. All statistical analyses were performed with SPSS for windows 13.0 statistics program (SPSS, Chicago, IL, USA). The results are presented as mean $\pm$ SD for continuous variables and proportion for categorical variables. A $p$ value of $<0.05$ was considered significant.

**Results**

***Subject characteristics, compliance and drop-out rate***

One hundred eighteen out of 120 volunteers (60 in the stanol group and 58 in the control group) completed the present study. Two volunteers in the control group dropped out due to time conflict with the scheduled visit to the research center and unwillingness to provide blood samples. Baseline characteristics are shown in Table 2. No significant differences existed in age, gender ratio, BMI, and serum lipoprotein levels between the stanol and control groups. Compliance with the present study was good; 98.7 and 99.1% of subjects in the control and stanol groups, respectively, consumed the provided soymilk daily during the present study period without any side effects.

**Table 2.** Baseline characteristics of the subjects in the 2 study groups[1]

| Variables | Control group (n = 58) | Stanol group (n = 60) |
|---|---|---|
| Gender (male/female) | 19/39 | 19/41 |
| Age (y) | 40.1 ± 8.4 | 39.8 ± 9.3 |
| Weight (kg) | 62.6 ± 12.8 | 61.4 ± 13.5 |
| Body mass index (kg/m$^2$) | 24.5 ± 4.1 | 24.0 ± 4.2 |
| Lipids (mmol/L) | | |
| Total cholesterol | 6.30 ± 0.80 | 6.33 ± 0.87 |
| LDL cholesterol | 4.16 ± 0.70 | 4.16 ± 0.71 |
| HDL cholesterol | 1.36 ± 0.29 | 1.36 ± 0.29 |
| Triglyceride | 1.55 ± 0.83 | 1.51 ± 0.88 |

[1] Values are means ± SD; No significant difference in all parameters between two groups, by unpaired Student's t-test

***Dietary intake***

Energy intake decreased marginally, but non-significantly, during the present study for both groups and involved all three dietary sources of energy. The proportion of each nutrient to overall energy intake was thus not affected (Table 3). At the end of the present study, control subjects tended to consume more carbohydrate and less protein than stanol subjects. There was no between-group difference in the proportion of fat consumed. Physical activity and other lifestyle behaviors remained stable. No significant weight change (less than 1 kg) was observed throughout the present study.

***Serum lipids and lipoproteins***

Significant reduction in serum TC and LDL-c levels were observed at week 2 in the stanol group and maintained until the end of the present study (Table 4). Only serum LDL-c level decreased significantly in the control group at week two, but rose back to almost their baseline level at week six (Table 4). A significant difference in percentage reduction of serum TC and LDL-c levels existed between stanol and control groups (Fig. 1). Serum HDL-c level dropped marginally in both groups at week two, but rose again to almost their baseline levels at week six. There was no significant change in serum triglyceride level for both within-group and between-group comparisons (Table 4).

***Serum lipid-soluble antioxidants***

Concentrations of lipid-soluble antioxidants were within normal reference limits in all subjects during the present study. Consumption of the plant stanol esters significantly lowered serum concentrations of α-tocopherol, β-cryptoxanthin, lycopene, and β-carotene (Table 5). After standardization of the lipid-soluble antioxidants for TC, changes in the ratios of α-tocopherol:TC and lycopene:TC between control and stanol groups were not significantly different. However, adjustment with TC, serum β-cryptoxanthin and β-carotene levels in the stanol group still decreased significantly from baseline at week six, and changes were still significantly different from the control group. In the control group, only serum retinol level decreased from baseline at week six, regardless of adjustment (Table 5).

**Table 3.** Nutrient intakes during the intervention in the 2 study groups[1]

| Nutrients | Control group (n = 58) | | | Stanol group (n = 60) | | |
|---|---|---|---|---|---|---|
| | 0 wk | 2 wk | 6 wk | 0 wk | 2 wk | 6 wk |
| Energy (kcal) | 1,313.2 ± 327.9 | 1,257.3 ± 399.4 | 1,269.3 ± 347.7 | 1,414.6 ± 447.7 | 1,314.0 ± 494.8 | 1,294.0 ± 350.9 |
| Carbohydrate (g/d) | 165.8 ± 49.1 | 164.9 ± 61.6 | 162.8 ± 45.7 | 180.4 ± 58.5 | 167.7 ± 69.0 | 161.3 ± 46.9 |
| Protein (g/d) | 56.5 ± 27.5 | 52.8 ± 21.3 | 49.8 ± 17.0 | 58.4 ± 24.7 | 57.1 ± 27.2 | 57.2 ± 18.5 |
| Fat (g/d) | 47.5 ± 15.4 | 43.3 ± 16.4 | 45.1 ± 16.5 | 51.6 ± 21.9 | 46.4 ± 25.0 | 46.6 ± 16.8 |
| Cholesterol (mg/d) | 229.2 ± 152.7 | 257.2 ± 125.6 | 239.5 ± 107.1 | 242.6 ± 140.7 | 246.8 ± 133.0 | 261.0 ± 128.3 |
| Energy distribution | | | | | | |
| Fat (%) | 32.5 ± 6.7 | 31.0 ± 6.6 | 31.1 ± 6.7 | 32.2 ± 6.6 | 31.2 ± 8.3 | 32.1 ± 5.9 |
| Carbohydrate (%) | 50.6 ± 8.8 | 52.3 ± 7.6 | 52.9 ± 7.0 | 51.5 ± 8.1 | 51.9 ± 9.7 | 50.1 ± 7.0 |
| Protein (%) | 16.9 ± 4.9 | 16.7 ± 4.4 | 16.0 ± 3.2 | 16.3 ± 3.3 | 17.3 ± 3.9 | 17.7 ± 3.2 |

[1] Values are means ± SD; No significant difference in all nutrient intakes between two groups at baseline, 2 wk and 6 wk, by repeated measure analysis of variance

**Table 4.** Serum lipids during the experimental period[1]

| Serum lipids (mmol/L) | Control group | | | Stanol group | | |
|---|---|---|---|---|---|---|
| | 0 wk | 2 wk | 6 wk | 0 wk | 2 wk | 6 wk |
| Total cholesterol | 6.30 ± 0.80 | 6.20 ± 0.84 | 6.26 ± 0.72 | 6.33 ± 0.87[a] | 5.75 ± 0.87[b] | 5.81 ± 0.85[b] |
| LDL cholesterol | 4.16 ± 0.70[a] | 3.92 ± 0.79[b] | 3.97 ± 0.70[ab] | 4.16 ± 0.71[a] | 3.57 ± 0.76[b] | 3.60 ± 0.74[b] |
| HDL cholesterol | 1.36 ± 0.30[ab] | 1.34 ± 0.32[a] | 1.39 ± 0.30[b] | 1.36 ± 0.27[ab] | 1.32 ± 0.31[a] | 1.39 ± 0.28[b] |
| Triglyceride | 1.55 ± 0.83 | 1.48 ± 1.02 | 1.55 ± 0.85 | 1.51 ± 0.88 | 1.36 ± 0.78 | 1.47 ± 0.84 |

[1] Values are means ± SD; For each row of each group, superscript containing no common alphabet denotes significant difference ($p < 0.05$), by repeated measure analysis of variance

**Table 5.** Serum fat-soluble antioxidants during the experimental period[1]

| | Concentration (μmol/L) | | Adjusted with TC (μmol/mmol) | |
|---|---|---|---|---|
| | Control group | Stanol group | Control group | Stanol group |
| Retinol | | | | |
| 0 wk | 1.85 ± 0.49 | 1.93 ± 0.39 | 0.29 ± 0.08 | 0.31 ± 0.07 |
| 6 wk | 1.73 ± 0.42 | 1.86 ± 0.44 | 0.27 ± 0.07 | 0.32 ± 0.08 |
| Change | -0.12 ± 0.22 | -0.07 ± 0.27 | -0.02± 0.04 | 0.01± 0.05* |
| ϒ-tocopherol | | | | |
| 0 wk | 2.96 ± 3.00 | 2.31 ± 2.09 | 0.48 ± 0.55 | 0.37 ± 0.35 |
| 6 wk | 3.40 ± 2.18 | 2.60 ± 2.05 | 0.54 ± 0.35 | 0.46 ± 0.38 |
| Change | 0.46 ± 3.45 | 0.29 ± 2.92 | 0.06 ± 0.59 | 0.09 ± 0.50 |
| α-tocopherol | | | | |
| 0 wk | 27.90 ± 4.82 | 26.97 ± 6.27 | 4.43 ± 0.73 | 4.30 ± 0.83 |
| 6 wk | 27.72 ± 4.59 | 23.11 ± 5.71 | 4.41 ± 0.70 | 4.01 ± 0.97 |
| Change | -0.20 ± 4.50 | -3.85 ± 5.84* | -0.02 ± 0.69 | -0.28 ± 0.93 |
| Lutein + Zeaxanthin | | | | |
| 0 wk | 0.68 ± 0.23 | 0.80 ± 0.26 | 0.11 ± 0.03 | 0.13 ± 0.04 |
| 6 wk | 0.75 ± 0.29 | 0.80 ± 0.35 | 0.12 ± 0.05 | 0.14 ± 0.05 |
| Change | 0.07 ± 0.28 | 0.002 ± 0.40 | 0.01 ± 0.04 | 0.009 ± 0.06 |
| β-cryptoxanthin | | | | |
| 0 wk | 0.68 ± 0.59 | 0.75 ± 0.70 | 0.11 ± 0.10 | 0.12 ± 0.11 |
| 6 wk | 0.86 ± 0.59 | 0.65 ± 0.37 | 0.14 ± 0.11 | 0.12 ± 0.07 |
| Change | 0.20 ± 0.38 | -0.10 ± 0.59* | 0.03 ± 0.06 | -0.007 ± 0.09* |
| Lycopene | | | | |
| 0 wk | 0.21 ± 0.13 | 0.23 ± 0.13 | 0.03 ± 0.02 | 0.04 ± 0.02 |
| 6 wk | 0.24 ± 0.16 | 0.19 ± 0.10 | 0.04 ± 0.03 | 0.03 ± 0.02 |
| Change | 0.03 ± 0.15 | -0.03 ± 0.09* | 0.006 ± 0.02 | -0.002 ± 0.02 |
| α-carotene | | | | |
| 0 wk | 0.08 ± 0.07 | 0.08 ± 0.07 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| 6 wk | 0.09 ± 0.09 | 0.07 ± 0.08 | 0.01 ± 0.01 | 0.01 ± 0.01 |
| Change | 0.01 ± 0.12 | -0.004 ± 0.03 | 0.002 ± 0.02 | 0.0004 ± 0.005 |
| β-carotene | | | | |
| 0 wk | 0.65 ± 0.33 | 0.65 ± 0.41 | 0.10 ± 0.05 | 0.10 ± 0.07 |
| 6 wk | 0.74 ± 0.38 | 0.54 ± 0.34 | 0.12 ± 0.07 | 0.09 ± 0.06 |
| Change | 0.09 ± 0.31 | -0.11 ± 0.22* | 0.02 ± 0.05 | -0.01 ± 0.03* |

[1] Values are means ± SD; TC = total cholesterol

Differences in changes between the groups were tested with an unpaired t-test, in which * denotes significant difference from control group

**Table 6.** Serum testosterone, estradiol, luteinizing hormones during the experimental period[1]

| Hormones | Control group | Stanol group |
|---|---|---|
| Testosterone[2] (ng/dL) | | |
| 0 wk | 400.78 ± 145.48 | 508.56 ± 182.1 |
| 6 wk | 413.33 ± 153.97 | 491.33 ± 184.0 |
| Estradiol[3] (pg/mL) | | |
| 0 wk | 111.55 ± 117.47 | 108.85 ± 128.31 |
| 6 wk | 137.10 ± 117.53 | 98.00 ± 93.83 |
| Luteinizing[4] (mIU/mL) | | |
| 0 wk | 14.26 ± 20.49 | 12.56 ± 24.33 |
| 6 wk | 14.66 ± 19.37 | 14.53 ± 29.79 |

[1] Values are means ± SD; No significant difference was observed either within group (Wilcoxon matched pairs test) or between control and stanol groups (Mann-Whitney test) in all parameters

[2] Testosterone was measured in a sub-sample of male subjects: n = 9 per group

[3] Estradiol was measured in a sub-sample of female subjects: n = 20 per group

[4] Luteinizing hormone was measured in a sub-sample of male and female subjects: n = 29 per group

### *Estradiol, testosterone, and luteinizing hormone*

Compared to baseline levels, serum estradiol, testosterone, and luteinizing hormones remained unchanged at week six (Table 6). No difference was observed between the control and stanol groups.

## Discussion

The cholesterol lowering effect of 2-g stanol in Thai volunteers is consistent with studies conducted in Western countries, both in the efficacy of stanol and the extent to which serum LDL-c level was lowered[9,21-24]. In the present study, serum TC and LDL-c concentrations decreased by about 8% and 14%, respectively, in mildly hypercholesterolemic subjects (Fig. 1). The reduction could be achieved by week two and maintained through to week six.

The decreases in TC and LDL-c were also in line with two studies conducted in Asia on different food products. A study in Japan[25] showed that consumption of 2 g/d plant stanols in a plant stanol ester spread could reduce LDL-c by 9.6% at week four. A second study in Korea[26] indicated that the same dose of 2 g plant stanols in a plant stanol ester low-fat yogurt lowered LDL-c 10% without affecting HDL-c and triglyceride concentrations. These results confirm that a minimal difference exists between high or low fat food sources in terms of their efficacy.

Serum LDL-c concentration in the control group also decreased at weeks two and six by 4% compared to baseline. This decline may be due to the inevitable tendency of volunteers to modify their eating behaviors at the beginning of the present study. As shown in Table 3, energy intake, especially from fat, tended to decrease at week two in both groups. This change in eating behavior may also help explain why serum HDL-c concentration dropped slightly at week two but rose to almost its baseline level at week six as subjects resumed their habitual eating pattern. Soy protein may partially explain the reduction of serum TC and LDL-c levels in both groups. However, the amount of soy protein found to achieve this effect was 3-185 mg[27], far higher than the approximately

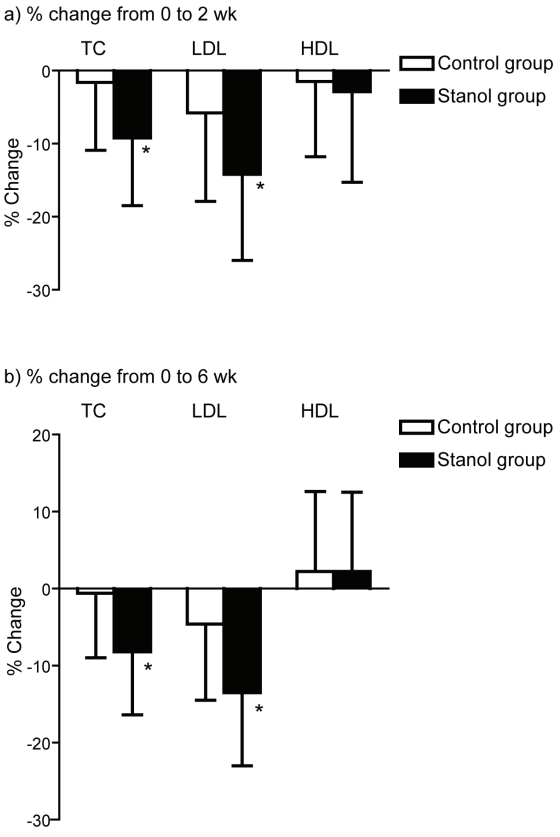


**Fig. 1** Comparison of percentage (%) change: a) 0-2 wk; b) 0-6 wk for total cholesterol (TC), low density cholesterol (LDL-c) and high density lipoprotein (HDL-c) between the control and stanol groups. Statistical significance was tested by independent t-test. Asterisk indicates $p < 0.05$ compared with % change of control group

0.9 mg (data not shown) contained in the soymilk used in the present study.

Because the eating behavior of volunteers in both groups changed to the effect that energy consumption attributable to fat was reduced, such a temporary behavioral change could explain in part the reduction of serum LDL-c in the stanol group. As a result, the absolute LDL-c-reducing capacity of stanol in the present study, derived by subtracting the 4.2% reduction in the control group from the 13.6% observed in the stanol group, was 9.4%.

Reduced absorption of fat-soluble nutrients can accompany reduced cholesterol absorption from stanol, whereby more lipophyllic nutrients are more likely to have altered absorption. In the present study, the concentrations of α-tocopherol, β-cryptoxanthin, lycopene, and β-carotene in the stanol group decreased significantly compared to the control group. Since these fat-soluble antioxidants are carried by lipoproteins, correction for changes in TC was performed. Results showed that the significant reduction in serum β-carotene and β-cryptoxanthin in the stanol group was still observed. Previous studies reported a drop[9,14,28,29] or no change[17,18,30,31] in serum α-tocopherol, lycopene, and β-carotene concentrations after stanol consumption. The inconsistent impact of stanol on these nutrients may be attributed to the difference in the daily amount of stanol consumed, the duration of stanol consumption, and the types and quantity of foods consumed.

As with most studies, the present study did not control the type and quantity of foods consumed by volunteers. Therefore, the possible effect of a change in eating behavior on the serum levels of these nutrients cannot be ruled out. Surprisingly, both adjusted and non-adjusted serum retinol concentrations in the control group significantly decreased at week six from baseline. Despite the limitations of the authors' food analysis software to verify the present assumption, the result could be due to subjects consuming less retinol-rich foods during the present study period. Concentrations of lipid-soluble antioxidants, however, were within normal reference limits in all subjects during the present study. In order to prevent decreased levels of fat-soluble antioxidants, daily consumption of good sources of these antioxidants, such as vegetables, fruits, and whole grains, should be encouraged.

Owing to the fact that cholesterol is a precursor for sex hormones, a hypothetical concern exists for the cholesterol-lowering effect of stanol on sex hormones. No adverse effects on sex hormone metabolism have thus far been demonstrated, except for the parenteral administration of large doses in experimental animals[19]. In the present study, sex hormones of randomly selected subjects were found to be unaffected by stanol. Small subsample size, possible inconsistent menstrual phases among female subjects notwithstanding the random-selection method, and short exposure to stanol may explain the nil finding. Another probable explanation is the cholesterol absorbed into the blood stream, together with the endogenous cholesterol production, was sufficient to help the body maintain its cholesterol homeostasis, as evidenced by a study on the effect of statin, a drug that lowers cholesterol through a different mechanism, on sex hormones[32]. The present study found no significant change in serum sex hormone levels in diabetic subjects after three months of statin administration.

In conclusion, the authors' findings suggest that daily consumption of 2-g stanol contained in a low-fat food vehicle (i.e. soymilk) within 30 minutes postprandially is effective in lowering serum LDL-c level by approximately 10 percent in Thais. Although it can be assumed that serum LDL-c concentration in Thais could remain low with long-term consumption of stanol, the effect of stanol consumption for a longer duration remains unknown. A reduction in the serum cholesterol level did not affect sex hormone balance. However, although a stanol-enriched food product is a suitable cholesterol-reduction strategy among Thai people and, by extrapolation, Asians, it may not be enough to warn stanol consumers of possible deficiencies in fat-soluble nutrients and to advise them to eat a diverse diet rich in these nutrients. Adding the nutrients to the stanol-enriched product may need to become mandatory to prevent undesired consequences.

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**Potential conflicts of interest**

None.

**References**

1. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001; 285: 2486-97.
2. Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, Franch HA, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. Circulation 2006; 114: 82-96.
3. Ostlund RE Jr, McGill JB, Zeng CM, Covey DF, Stearns J, Stenson WF, et al. Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans. Am J Physiol Endocrinol Metab 2002; 282: E911-6.
4. Ikeda I, Sugano M. Inhibition of cholesterol absorption by plant sterols for mass intervention. Curr Opin Lipidol 1998; 9: 527-31.
5. Wilson MD, Rudel LL. Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. J Lipid Res 1994; 35: 943-55.
6. Heinemann T, Kullak-Ublick GA, Pietruck B, von Bergmann K. Mechanisms of action of plant sterols on inhibition of cholesterol absorption. Comparison of sitosterol and sitostanol. Eur J Clin Pharmacol 1991; 40 (Suppl 1): S59-63.
7. 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.
8. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 2003; 78: 965-78.
9. Cater NB, Grundy SM. Lowering serum cholesterol with plant sterols and stanols. Historical perspectives. In: Nguyen TT, editor. New developments in the dietary management of high cholesterol. (Postgraduate medicine special report). Minneapolis, MN: McGraw-Hill; 1998: 6-14.
10. Piironen V, Toivo J, Puupponen-Pimia R, Lampi AM. Plant sterols in vegetables, fruits, and berries. J Sci Food Agric 2003; 83: 330-7.
11. Food and Drug Administration, Department of Health and Human Services. Food labeling: health claims: plant sterol/stanol esters and coronary heart disease. Fed Register 2000; 65: 54685-739.
12. O'Neill FH, Sanders TA, Thompson GR. Comparison of efficacy of plant stanol ester and sterol ester: short-term and longer-term studies. Am J Cardiol 2005; 96: 29D-36D.
13. 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.
14. 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.
15. Colgan HA, Floyd S, Noone EJ, Gibney MJ, Roche HM. Increased intake of fruit and vegetables and a low-fat diet, with and without low-fat plant sterol-enriched spread consumption: effects on plasma lipoprotein and carotenoid metabolism. J Hum Nutr Diet 2004; 17: 561-9.
16. Raeini-Sarjaz M, Ntanios FY, Vanstone CA, Jones PJ. No changes in serum fat-soluble vitamin and carotenoid concentrations with the intake of plant sterol/stanol esters in the context of a controlled diet. Metabolism 2002; 51: 652-6.
17. 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.
18. Tikkanen MJ. Plant sterols and stanols. Handb Exp Pharmacol 2005; 215-30.
19. Mussner MJ, Parhofer KG, Von Bergmann K, Schwandt P, Broedl U, Otto C. Effects of phytosterol ester-enriched margarine on plasma lipoproteins in mild to moderate hypercholesterolemia are related to basal cholesterol and fat intake. Metabolism 2002; 51: 189-94.
20. Miettinen TA, Puska P, Gylling H, Vanhanen H, Vartiainen E. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. N Engl J Med 1995; 333: 1308-12.
21. Bhattacharya S. Therapy and clinical trials: plant sterols and stanols in management of hypercholesterolemia: where are we now? Curr Opin Lipidol 2006; 17: 98-100.
22. Noakes M, Clifton PM, Doornbos AM, Trautwein

EA. Plant sterol ester-enriched milk and yoghurt effectively reduce serum cholesterol in modestly hypercholesterolemic subjects. Eur J Nutr 2005; 44: 214-22.
23. Plat J, Mensink RP. Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. Am J Cardiol 2005; 96: 15D-22D.
24. Devaraj S, Jialal I, Vega-Lopez S. Plant sterol-fortified orange juice effectively lowers cholesterol levels in mildly hypercholesterolemic healthy individuals. Arterioscler Thromb Vasc Biol 2004; 24: e25-8.
25. Homma Y, Ikeda I, Ishikawa T, Tateno M, Sugano M, Nakamura H. Decrease in plasma low-density lipoprotein cholesterol, apolipoprotein B, cholesteryl ester transfer protein, and oxidized low-density lipoprotein by plant stanol ester-containing spread: a randomized, placebo-controlled trial. Nutrition 2003; 19: 369-74.
26. Hyun YJ, Kim OY, Kang JB, Lee JH, Jang Y, Liponkoski L, et al. Plant stanol esters in low-fat yogurt reduces total and low-density lipoprotein cholesterol and low-density lipoprotein oxidation in normocholesterolemic and mildly hyper-cholesterolemic subjects. Nutr Res 2005; 25: 743-53.
27. Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr 2005; 81: 397-408.
28. Gylling H, Miettinen TA. Cholesterol reduction by different plant stanol mixtures and with variable fat intake. Metabolism 1999; 48: 575-80.
29. Hallikainen MA, Uusitupa MI. Effects of 2 low-fat stanol ester-containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects. Am J Clin Nutr 1999; 69: 403-10.
30. Mensink RP, Ebbing S, Lindhout M, Plat J, van Heugten MM. Effects of plant stanol esters supplied in low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. Atherosclerosis 2002; 160: 205-13.
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. Santini SA, Carrozza C, Lulli P, Zuppi C, CarloTonolo G, Musumeci S. Atorvastatin treatment does not affect gonadal and adrenal hormones in type 2 diabetes patients with mild to moderate hypercholesterolemia. J Atheroscler Thromb 2003; 10: 160-4.

## *การลดลงของแอลดีแอลคอเลสเตอรอลในคนไทยที่มีคอเลสเตอรอลสูงจากการดื่มนมถั่วเหลืองเสริมพลานท์สเตนอล*

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***วัตถุประสงค์:*** *เพื่อศึกษาประสิทธิผลของนมถั่วเหลืองเสริมสเตนอลต่อการเปลี่ยนแปลงของแอลดีแอลคอเลสเตอรอล วิตามินที่ละลายในไขมัน และฮอร์โมนเพศในอาสาสมัครคนไทย*

***วัสดุและวิธีการ:*** *รูปแบบการศึกษาเป็นแบบสุ่มโดยการปกปิดทั้งสองด้าน ทำการศึกษาในอาสาสมัครคนไทยที่มีคอเลสเตอรอลในเลือดสูง 120 คน แบ่งเป็นกลุ่มควบคุมที่ได้รับนมถั่วเหลืองธรรมดา และกลุ่มสเตนอลที่ได้รับนมถั่วเหลืองเสริมสเตนอลวันละ 2 กรัม โดยที่อาสาสมัครทั้ง 2 กลุ่ม มีระดับไขมันในเลือดและดัชนีมวลกายเบื้องต้นไม่แตกต่างกัน อาสาสมัครดื่มนมถั่วเหลืองดังกล่าวทุกวันหลังอาหารเป็นระยะเวลา 6 สัปดาห์ มีการตรวจวัดการเปลี่ยนแปลงของไขมันในเลือดในสัปดาห์ที่ 0, 2, 6 และตรวจวัดวิตามินที่ละลายในไขมันและฮอร์โมนเพศในสัปดาห์ที่ 0 และ 6*

***ผลการศึกษา:*** *กลุ่มสเตนอลมีค่าเฉลี่ยของการลดลงของ total cholesterol 8.2% ขณะที่กลุ่มควบคุมลดลง 0.6% สำหรับ LDL cholesterol มีค่าลดลงทั้งกลุ่มควบคุมและกลุ่มสเตนอลในสัปดาห์ที่ 2 แต่มีการลดลงเพิ่มขึ้นในสัปดาห์ที่ 6 เฉพาะกลุ่มสเตนอลซึ่งลดลงได้ 13.5% เทียบกับกลุ่มควบคุมที่ลดลง 4.6% ($p < 0.0001$) ในสัปดาห์ที่ 6 พบว่าระดับ $\beta$-cryptoxantene and $\beta$-carotene ลดลง แต่ไม่มีการเปลี่ยนแปลงของระดับฮอร์โมนเพศในทั้งสองกลุ่ม*

***สรุป:*** *การบริโภคนมถั่วเหลืองเสริมสเตนอลเป็นระยะเวลา 6 สัปดาห์ ทำให้ LDL cholesterol ลดลง โดยที่ไม่มีผลต่อการเปลี่ยนแปลงของฮอร์โมนเพศ แต่อาจทำให้ระดับวิตามินที่ละลายในไขมันลดลงได้ ถ้ามีการบริโภคจากอาหารไม่เพียงพอ*