Customary Use of Plant Sterol and Plant Stanol Enriched Margarine Is Associated with Changes in Serum Plant Sterol and Stanol Concentrations in Humans¹,²

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Abstract

The consumption of products enriched with plant sterol or stanol esters lowers serum total and LDL-cholesterol concentrations, thereby most likely reducing the risk of coronary heart disease. However, using plant sterol (not plant stanol) enriched products elevates serum plant sterol concentrations in humans. This may be unwanted because health effects of elevated serum plant sterol concentrations are still controversial. Within postlaunch monitoring of functional foods, we compared serum plant sterol and plant stanol concentrations among users of plant sterol (n = 67) or plant stanol (n = 13) enriched margarines with those of matched nonusers (n = 81) in the ongoing Dutch Doetinchem cohort study. Subjects (aged 29–67 y) were examined in 1994–1998 (before the introduction of enriched margarines) and re-examined in 1999–2003. Serum concentrations of plant sterols and stanols were measured in samples from nonfasting subjects by GLC-MS. Intake of plant sterol was 1.1 ± 0.2003 g/d and was associated with a decrease of serum total cholesterol concentration of 0.25 ± 0.091 mmol/L (4%, P < 0.05), a change that differed (P < 0.05) from the nonsignificant increase in nonusers (+2%, 0.12 ± 0.078 mmol/L, P = 0.16). Cholesterol-standardized serum sitosterol and campesterol increased in plant sterol users by 22% (P < 0.0001) and 103% (P < 0.0001), respectively. Cholesterol-standardized serum sitostanol and campestanol increased in plant stanol users by 197% (P = 0.02) and 196% (P = 0.01). To our knowledge, these data are the first to show changes in serum cholesterol, plant sterol, and plant stanol concentrations after (long-term) consumption of plant sterol and stanol enriched margarines in a free-living population in a nonexperimental setting. Whether the increased serum sterol concentrations result in adverse side effects needs to be investigated in future postlaunch monitoring studies. J. Nutr. 137: 1301–1306, 2007.

Introduction

The consumption of products enriched with plant sterol or stanol esters lowers total and LDL cholesterol concentrations, thereby most likely reducing the risk of coronary heart disease (1). The European Scientific Committee on Food (SCF) has authorized the use of plant sterol and plant stanol esters in a number of foods from 1999 onwards (2). Upon marketing approval, several requirements were specified, among others, regarding postlaunch monitoring. It was subsequently concluded that the intake of plant sterol–enriched margarine in the general free-living population was lower than anticipated and that there were no unpredicted (side) effects observed, so consumption would be safe (2,3).

In numerous well-controlled human intervention trials, the consumption of plant sterol–enriched products increased serum plant sterol concentrations in human subjects, whereas the use of plant stanol–enriched products decreased serum plant sterol concentrations (4–7). Based on recent scientific understandings, there is now concern about the possible unwanted effects of elevated concentrations of plant sterols in serum. Whether such concerns also apply to elevated plant stanol concentrations has, unfortunately, hardly been evaluated (8).

One of the concerns is the potential atherogenicity of elevated serum plant sterol concentrations. The first indication is the presence of coronary atherosclerosis in sitosterolemic patients, characterized by severely elevated serum plant sterol concentrations.
mostly in the absence of hypercholesterolemia, which suggests that high circulating levels of plant sterols may be atherogenic (9–11). In addition, several epidemiological studies support this assumption (12–16). However, as reviewed by Patel and Thompson (17), results of these studies are not conclusive and 2 studies do not support the assumption (18,19). The strongest indication of plant sterols being atherogenic can be found in the 4S study. In this study, simvastatin treatment, which elevates cholesterol-standardized serum plant sterol concentrations, was not associated with a reduction in mortality in subjects that responded with the largest elevation in serum plant sterol concentrations (14). Therefore, monitoring the side effects of consuming plant sterol–enriched products in statin users may be of utmost importance. Unfortunately, long-term trials or epidemiological studies of sufficient size and including firm endpoints are not yet available (17,20).

In the first postlaunch monitoring attempts performed by the manufacturer of plant sterol–enriched margarines, it was concluded that the general intake of these products did not induce unpredicted (side) effects (2,3). However, an assessment of serum plant sterol concentrations was not included in that program. In view of the suggested atherogenic effect of elevated serum plant sterol concentrations, ongoing monitoring of consumers within the community seems prudent. Under this postlaunch monitoring heading we analyzed serum plant sterol, plant stanol and cholesterol concentrations among users of plant sterol or plant stanol–enriched margarines and compared them to matched nonusers in an ongoing free-living Dutch cohort study over a 5-y follow-up period.

**Methods**

The Dutch Doetinchem study, a longitudinal cohort study, investigates changes in lifestyle and (biological) risk factors of chronic diseases within adult individuals over time in consecutive 5-y intervals. Complete data were available for 4505 subjects (2138 males and 2367 females), aged 26–70 y. Measurements performed between 1994–1998 (before the introduction of plant sterol–enriched margarines) were used as baseline measurements and measurements performed between 1999–2003 were used as 5-y follow-up measurements. Details of the overall cohort study have been described elsewhere (21). The study was approved according to the guidelines of the Helsinki Declaration by the external Medical Ethical Committee of the Dutch TNO Research Institute.

In short, the cohort study included, among others, a general questionnaire containing questions on demographic and lifestyle factors (e.g., smoking, physical activity), subjective health, and disease prevalence. Also, a validated FFQ, developed for Dutch cohorts within the context of the European Prospective Investigation into Cancer and Nutrition (EPIC), asked for usual food intake during the previous year (22). This FFQ contained one open question on the brand name of margarine used, by which plant sterol and plant stanol enriched margarine users could be selected. The amount of margarine used was calculated by multiplying the number of slices of bread consumed daily by the amount of spread on a slice that was estimated by a series of photographs.

A physical examination took place at the regional public health service (in Dutch, “GGD”), which included anthropometric measurements (weight, height) and measurements of blood pressure. BMI was calculated as kg/m². In addition, nonfasting blood samples were obtained, using a standardized protocol, for analyses of serum concentrations of total and HDL cholesterol, plant sterols and stanols, and markers for endogenous cholesterol synthesis (23). Serum samples were frozen at −20°C for storage and transported to the laboratory within 3–12 wk after collection. Part of each serum sample was stored at −86°C for future analyses. Cholesterol determinations were performed in the Lipid Reference Laboratory of the University Hospital in Rotterdam. At baseline, plasma total cholesterol was measured with the CHOD-PAP-method (Boehringer) (24) and HDL cholesterol was determined after precipitation of apob-containing lipoproteins with phosphotungstic acid/MgCl₂ (Boehringer) (25). At the 5-y follow-up, serum total cholesterol was measured, and HDL cholesterol was determined using a direct method with lyophilized reagent. Beginning in 2002, a homogen liquid second generation assay was used for HDL cholesterol determination (26). This method was calibrated against the baseline method used in the Lipid Reference Laboratory; there was no divergence in the results on population level in mean HDL cholesterol concentrations (21).

In 2005, baseline and 5-y follow-up serum concentrations of plant sterols (campesterol and sitosterol) and stanols (campestanol and sitostanol) and 2 markers of endogenous cholesterol synthesis (lathosterol and desmosterol) were analyzed by GLC or GLC-MS–selected ion-monitoring in the Department of Clinical Pharmacology, University of Bonn, according to standard protocols (27). The detection limits were <0.26 nmol/L for plant sterols/stanols, lathosterol and desmosterol; the CV between and within runs were <5%. Storage stability of sterols, stanols, and cholesterol precursors had been evaluated earlier in this laboratory and absolute concentrations decreased by maximum of 2% after 5 y. Cholesterol-standardized concentrations were calculated by dividing the serum plant sterol concentrations by the enzymatically measured cholesterol concentrations.

For our study, subjects were stratified into plant sterol or plant stanol enriched margarine users (n = 84) and matched nonusers (n = 84) based on the follow-up data, as the plant stanol and plant sterol enriched margarines were available on the Dutch market from 1999 and 2000 onwards, respectively. Subjects were matched for gender, age, BMI, baseline total plasma cholesterol concentration, and self-reported use of cholesterol-lowering medication. Baseline and follow-up data on serum plant sterol, plant stanol, lathosterol and desmosterol concentrations for 80 users of plant sterol or plant stanol–enriched margarine and 81 matched nonusers were available for further statistical analyses.

**Statistical methods**

General characteristics among users and nonusers of plant sterol or plant stanol–enriched margarines were calculated for descriptive purposes. Statistical differences between users and nonusers were tested using the student’s t test for continuous variables and χ² test for nominal variables.

Mean ± SD serum plant sterol, plant stanol, lathosterol and desmosterol concentrations, as well as their cholesterol-standardized concentrations, were calculated for both users and nonusers. As plant sterols are more absorbed than plant stanols, a distinction among users was made between users of plant sterols (i.e., consumers of Becel pro.activ margarine) and plant stanols (i.e., consumers of Benecol margarine). A paired t test was used to compare differences between baseline and follow-up values within each of the 3 groups. The difference between groups was tested using ANOVA with Bonferroni correction for multiple comparisons (for variables with equal variances) or the Kruskal Wallis test (for variables with unequal variances). A P-value of <0.05 was considered significant. Statistical analyses were performed using SAS, version 9.1 (SAS Institute).

**Results**

The general characteristics of plant sterol or plant stanol enriched margarine users and nonusers did not differ except for smoking, systolic blood pressure, and the prevalence of ever being diagnosed with a high cholesterol level (Table 1). The mean daily consumption of the plant sterol–enriched margarine was 14 g/d (range 1–38 g/d), which resulted in a mean plant sterol intake of −1.1 ± 0.6 g/d. The mean plant stanol–enriched margarine intake was 9 g/d (range 1–21 g/d), resulting in −0.6 ± 0.4 g/d of plant stanols.

Subjects were successfully matched for baseline plasma total cholesterol concentrations, which did not differ between users (6.23 ± 0.97 mmol/L, n = 80) and nonusers of enriched margarines (5.70 ± 1.08 mmol/L, n = 81). However, when plant sterol users (n = 67) and plant stanol users (n = 13) were analyzed separately, the baseline plasma total cholesterol concentration...
differed significantly between the plant sterol users and all nonusers (Table 2).

Consumption of plant sterol enriched margarine was associated with a significant 4% reduction of serum total cholesterol concentrations in 5 y (Table 2).

**Serum plant sterols.** In users of plant sterol–enriched margarine, cholesterol-standardized sitosterol concentrations tended to decrease during the 5 y (18%; \( P = 0.06 \)) and the cholesterol-standardized campesterol concentration tended to decrease by 11% (\( P = 0.11 \)); these changes did not differ from those of all nonusers (Table 2). In users of plant stanol-enriched margarine, the cholesterol-standardized sitosterol concentration tended to decrease during the 5y study (\( P = 0.06 \)) and the cholesterol-standardized campesterol concentration tended to decrease by 11% (\( P = 0.11 \)); these changes did not differ from those of all nonusers.

**Serum plant stanols.** In users of plant stanol–enriched margarine, cholesterol-standardized sitostanol concentrations increased by 197% (\( P = 0.02 \)) and cholesterol-standardized campestanol concentrations increased by 196% (\( P = 0.01 \)), resulting in greater changes in concentration than in all nonusers (\( P < 0.05 \)) (Table 2).

The ratios of desmosterol and lathosterol to cholesterol are used as markers of endogenous cholesterol synthesis (28) (Table 2). In users of plant sterol–enriched margarine, both cholesterol-standardized desmosterol and lathosterol concentrations increased by 25% (\( P < 0.0001 \)) and 15% (\( P = 0.006 \)) between baseline and 5-y follow-up, respectively. The increase in cholesterol-standardized desmosterol resulted in greater changes in concentration than in all nonusers (\( P < 0.05 \)) (Table 2). Interestingly, use of plant stanol esters was not associated with a compensatory increase in endogenous cholesterol synthesis.

**Discussion**

Numerous studies have examined changes in concentrations of serum lipoproteins and plant sterols in well-controlled short-term intervention studies. One of the first reports on this dates from 1976 (29). In line with these findings, we showed that customary use of plant sterol–enriched margarines under free-living conditions is also associated with significantly lower serum total cholesterol concentrations, and increases in cholesterol-standardized serum sitosterol and campesterol concentrations. In addition, customary use of plant stanol–enriched margarines results in a comparable reduction of total serum cholesterol concentrations as well as a decrease in cholesterol-standardized serum sitosterol and campesterol concentrations, whereas serum cholesterol-standardized plant stanol concentrations were elevated.

The data presented in this paper are observations in free-living conditions. This implies that it represents what is happening in a nonexperimental setting but the data are also prone to diluting effects and confounding, e.g., diet or behavioral changes during study follow-up. One example of a diluting effect is the fact that, generally, users of the enriched margarines consumed less than the 20 g/d of margarine recommended by the manufacturers. Nevertheless, our results are largely in line with observations from controlled clinical trials, discussed below. The cholesterol-lowering effect of enriched margarines was greater than expected in this study, based on the sole use of enriched margarines. This was due to the fact that users of cholesterol-lowering medication were also included in the 2 groups of enriched margarine users: there were 8 medication (statin) users in the plant sterol group and 4 medication users in the plant stanol group. In a recently published paper that used the same study population, a 5-y difference in total cholesterol concentrations of −0.08 mmol/L was found in only enriched margarine users and −1.34 mmol/L in combination users (statins and enriched margarine) (30).

In this study, cholesterol-standardized serum sitosterol concentrations increased by 22% with long-term plant sterol consumption and decreased by 18% with plant stanol consumption, whereas standardized campesterol concentrations increased by 103% with consumption of sterol–enriched margarine and decreased by 11% with plant stanol margarine use. O’Neill et al. (31) described a mean change in standardized concentrations of campesterol of 40% and sitosterol of 20% with a plant sterol consumption of 1.6 g/d for 2 mo, whereas plant stanol consumption of 2.6 g/d for 2 mo resulted in a decrease of 25–35%. Hallikainen et al. (32) found a 50–80% increase in standardized serum plant sterol concentrations with plant stanol consumption (2 g/d for 10 wk, hypercholesterolemic subjects) and a 25–30% decrease with plant stanol consumption. A long-term study by Hendriks et al. (33) showed a 92% increase in standardized campesterol concentrations and a 33% increase in standardized sitosterol concentrations after 1 y of consuming 1.6 g/d of plant stanols.
sterols. Thus, the large increase in campesterol concentrations compared with the increase of sitosterol concentrations in our study (103 vs. 22%) is also seen in clinical trials. In some trials, standardized campesterol concentrations doubled with margarine consumption (1,7,31,32).

Cholesterol standardized sitostanol concentrations increased by 197% with long-term plant stanol consumption in our study, whereas cholesterol-standardized campesterol concentrations increased by 196%. These changes are comparable with changes found in clinical trials; e.g., Hallikainen et al. (4) found a 53% increase in cholesterol-standardized sitostanol concentrations and a 166% increase in standardized campesterol concentrations in hypercholesterolemic subjects consuming 2.4 g of plant stanol esters for 4 wk. In an 8-wk trial, consumption of 2 g/d of plant stanol ester by healthy volunteers resulted in an increase in standardized sitostanol concentration of 500% and a 200% increase in campesterol concentrations (34).

Although our results are comparable to clinical data published earlier, there are also some differences. The main observation is that the relative changes in serum plant sterol concentrations are achieved by a lower customary dietary intake. Naumann et al. (7) estimated, from several short-term intervention studies, that the cholesterol-standardized serum plant sterol concentrations would increase by ~11–13% (sitosterol) and ~20–22% (campesterol) per gram of daily plant sterol intake. It is difficult to explain this deviation. The difference between the estimated effect by Naumann et al. and the observed effect in our present study might be caused by 1) unregistered changes in eating patterns, 2) the inclusion of medication users who might have a higher absorption rate, and 3) possible other confounding variables in customary settings that are unknown to us at this moment (e.g., other interfering drugs). Due to these issues especially, findings from controlled intervention studies regarding the effects of plant sterols, might, in some cases, be extrapolated to the free-living population, but this may not be correct in all cases. Because there are no reference values for normal values of serum plant sterols, we compared only relative changes. It was not appropriate to compare absolute figures because different analytical techniques vary considerably and act as strong confounders (35).

The clinical implication of high serum plant sterol concentrations, if any, is still unclear and under active investigation (17). Serum plant sterols might be considered as biomarkers for atherosclerosis (like cholesterol), but whether they have their own intrinsic activity is still unknown (20). We have shown in the present study, however, that elevations in controlled trials also occur in a free-living population over a 5-y follow-up period.

Some limitations of the current study should be considered. Among the total number of participants in our cohort, the number of enriched margarine users was small (n = 84, 2% of the total cohort), especially with regard to plant stanol ester–enriched margarine (n = 13). This low number of plant stanol users, compared with the number of plant sterol users, is a representative reflection of the availability of these products in the Netherlands. Therefore, a lack of power could be a reason for the nonsignificant changes found in, for example, the cholesterol-standardized sitosterol and campesterol concentrations in plant stanol users. Also, the matching of baseline cholesterol concentrations was based on users and nonusers and not specified to plant sterol and plant stanol users. As a result, the baseline

| TABLE 2 | Baseline concentrations and changes over 5 y in serum cholesterol, plant sterols, and plant stanols in users of plant sterol, or plant stanol–enriched margarine and in matched nonusers1 |
|-----------------------------------------------|-----------------|-----------------|-----------------|
|                                  | Plant sterol users, n = 67 | Plant stanol users, n = 13 | Nonusers (controls), n = 81 |
| Total cholesterol, mmol/L | Baseline 6.24 ± 0.93abc | 6.17 ± 1.22abc | 5.70 ± 1.08ab |
|                              | Change −0.25 ± 0.91abc | −0.47 ± 1.08abc | 0.12 ± 0.78ab |
| HDL cholesterol, mmol/L    | Baseline 1.38 ± 0.39 | 1.30 ± 0.37 | 1.29 ± 0.34 |
|                              | Change 0.00 ± 0.18 | 0.05 ± 0.26 | 0.00 ± 0.20 |
| Total/HDL cholesterol, mmol | Baseline 4.88 ± 1.56 | 5.12 ± 1.70 | 4.75 ± 1.59 |
|                              | Change −0.25 ± 1.09 | −0.37 ± 1.08 | 0.05 ± 1.01 |
| Sitosterol, μmol/L          | Baseline 8.45 ± 3.47abc | 7.09 ± 2.17abc | 6.82 ± 2.89ab |
|                              | Change 1.36 ± 3.08abc | −1.67 ± 2.08abc | −0.20 ± 2.45ab |
| Campesterol, μmol/L         | Baseline 136 ± 53 | 119 ± 41 | 124 ± 56 |
| 10 μmol/mmol cholesterol    | Change 30 ± 55ab | −22 ± 40b | −5.8 ± 43b |
| Campesterol, μmol/L         | Baseline 107.77 ± 4.86 | 9.58 ± 3.36 | 9.13 ± 3.72 |
| 10 μmol/mmol cholesterol    | Change 9.76 ± 6.72bc | −1.52 ± 1.85bc | 0.92 ± 1.65bc |
| Sitostanol, μmol/L          | Baseline 172 ± 73 | 157 ± 46 | 163 ± 65 |
| 10 μmol/mmol cholesterol    | Change 177 ± 126bc | −17 ± 36b | 14 ± 61bc |
| Sitostanol, μmol/L          | Baseline 0.16 ± 0.05a | 0.14 ± 0.04b | 0.14 ± 0.04b |
| 10 μmol/mmol cholesterol    | Change −0.03 ± 0.08bc | 0.24 ± 0.34ab | −0.02 ± 0.04ab |
| Campesterol, μmol/L         | Baseline 2.56 ± 0.73 | 2.30 ± 0.76 | 2.56 ± 0.94 |
| 10 μmol/mmol cholesterol    | Change −0.32 ± 1.24bc | 4.53 ± 6.39bc | −0.40 ± 0.80bc |
| Desmostanol, μmol/L         | Baseline 0.14 ± 0.04 | 0.13 ± 0.02 | 0.13 ± 0.03 |
| 10 μmol/mmol cholesterol    | Change 0.06 ± 0.07bc | 0.22 ± 0.28ab | 0.02 ± 0.05ab |
| Lathosterol, μmol/L         | Baseline 2.24 ± 0.61 | 2.12 ± 0.54 | 2.28 ± 0.66 |
| 10 μmol/mmol cholesterol    | Change 1.17 ± 1.25bc | 4.16 ± 5.21bc | 0.42 ± 0.95bc |
| 10 μmol/mmol cholesterol    | Change 75 ± 20 | 75 ± 22 | 70 ± 18 |
| Lathosterol, μmol/L         | Baseline 19 ± 17bc | 14 ± 19b | 9 ± 16bc |
| 10 μmol/mmol cholesterol    | Change 118 ± 48 | 123 ± 54 | 120 ± 47 |
| Lathosterol, μmol/L         | Baseline 18 ± 51* | 6 ± 50 | 2 ± 42 |

1 Values are means ± SD. Means in a row with superscripts without a common letter differ, *P < 0.05. *Change, *P < 0.05.
cholesterol concentrations between plant sterol users and non-users differed significantly ($P = 0.05$). Furthermore, a 5-y change in serum plant sterol and stanol concentrations was calculated in this study, but it is unknown whether the enriched margarines were used consistently during the whole period. The FFOQ is administered once every 5 y in the Doetinchem cohort study, and there are no data available per year.

In conclusion, to our knowledge, these data are the first to show changes in serum cholesterol and plant sterol and plant stanol concentrations after the long-term consumption of plant sterol and stanol–enriched margarines in a free-living population in a nonexperimental setting. Whether these changes are potentially hazardous and counteract the achieved beneficial effect of lowering serum total cholesterol concentrations should be investigated in future long-term (postlaunch) monitoring studies in the free-living population. Also, we stress the relevance of devoting more attention to the potential effects of elevated cholesterol-standardized serum plant sterols and stanols in statin users and in subjects with a family history of coronary heart disease, as statins may increase plant sterol concentrations, and people with a family history of coronary heart disease may be prone to higher concentrations. Data from these monitoring studies can be used in future risk-benefit analyses of plant sterol or stanol–enriched products to assess their overall effect in the population.

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Literature Cited


