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# Consuming Functional Foods Enriched with Plant Sterol or Stanol Esters for 85 Weeks Does Not Affect Neurocognitive Functioning or Mood in Statin-Treated Hypercholesterolemic Individuals[1–3]

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## Abstract

Recent animal and human studies have shown that plant sterols and stanols, which are used as functional food ingredients to lower increased LDL cholesterol concentrations, pass the blood-brain barrier. Whether this affects neurocognitive functioning and mental well-being in humans has, to our knowledge, never been investigated. The aim of the present study was therefore to examine the effects of long-term plant sterol or stanol consumption on neurocognitive functioning and mood in a randomized, double-blind, placebo-controlled dietary intervention trial. To this end, hypercholesterolemic individuals, aged 43–69 y, receiving stable statin treatment were randomly assigned to an 85-wk supplementation with margarines enriched with plant sterol esters (2.5 g/d), plant stanol esters (2.5 g/d), or placebo. At baseline and at the end of the intervention period, all participants underwent a cognitive assessment. In addition, subjective cognitive functioning and mood were assessed by means of questionnaires (Cognitive Failure Questionnaire and depression subscale of the Symptom Checklist 90, respectively). Long-term supplementation with plant sterol or stanol esters did not affect cognitive performance (memory, simple information processing speed, complex information processing speed, Letter-Digit Substitution test performance), subjective cognitive functioning, or mood. In conclusion, the present results indicate that long-term use of plant sterols or stanols at recommended intakes of 2.5 g/d does not affect neurocognitive functioning or mood in hypercholesterolemic individuals receiving statin treatment. J. Nutr. 139: 1368–1373, 2009.

## Introduction

Plant sterols and their saturated derivates, the plant stanols, which are primarily found in vegetables, vegetable oils, fruits, and cereals (1,2), are known for their cholesterol-lowering properties (3–5). Because of their effects on intestinal cholesterol absorption, these compounds are incorporated into the so-called functional foods (6).

The use of foods enriched with plant sterols and stanols, e.g. as an adjunctive cholesterol-lowering treatment in hypercholesterolemic patients receiving statin therapy (7), is generally considered safe (8,9). Only a relatively small fraction of plant sterols (0.4–3.5%) and stanols (0.02–0.3%) is absorbed in the intestine and therefore the serum levels of these compounds are rather low (10,11). Repeat-dose toxicology studies in rats have indicated that dietary intake of plant sterols or stanols up to 4 g/(kg · d), which is equivalent to ~280 g/d in humans (i.e. ~100 times higher than the recommended intake of 2.5 g/d) (12,13), does not have any adverse physiological effects (14,15). Because intestinal absorption of sterols is similar in rats and humans (10,16,17), it seems legitimate to extrapolate these results to the human population.

So far, no animal or human studies have, to our knowledge, assessed the possible effects of supplementation with plant sterol- or stanol ester-enriched foods on neurocognitive functioning and mental well-being, because it was long thought that circulating plant sterols and stanols could not pass the blood-brain barrier. This notion was primarily based on their structural resemblance to cholesterol, which does not enter the brain from circulation

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but is produced in situ by the central nervous system (18,19). Recent studies, however, including a human postmortem study, have demonstrated the presence of plant sterols and stanols in the brain (20–26). Whereas plant sterol and stanol concentrations in the brain normally range from 10 to 80 ng/mg tissue (21–26), supplementation with these compounds may increase their brain concentrations by . 200% (20,23). In addition, dysfunction of the blood-brain barrier as, e.g., in Alzheimer's disease, may also result in an increased influx of plant sterols and stanols from the blood stream into the brain (21,24,25).

Elevated levels of plant sterols and stanols in the central nervous system may have important consequences for brain functioning. Plant sterols and stanols are easily incorporated into cellular membranes, which may change their functional properties (27) and reduce their fluidity (28), thereby affecting (synaptic) signal transduction in the brain. Alternatively, plant sterols and stanols might also influence brain functioning by means of their cholesterol-lowering actions. Because cholesterol is the main lipid constituent of cellular membranes and myelin (18,19) and plays an important supportive role in signal transduction processes in the brain (29,30), a reduction in brain cholesterol may have a negative impact on neurocognitive functioning (31).

This study examined the consequences of long-term consumption of plant sterol and stanol esters on neurocognitive functioning and mood in statin-treated hypercholesterolemic individuals in a randomized, double-blind, placebo-controlled dietary intervention trial. Because supplementation with plant sterols and stanols as adjunctive cholesterol-lowering treatment typically involves consumption for long periods of time, the trial duration was 85 wk.

## Methods

*Participants.* The present study was part of a larger study investigating the long-term effects of combining statin treatment with plant sterol- or stanol ester-enriched functional foods on markers of endothelial dysfunction and arterial stiffness (32; A. de Jong, J. Plat, A. Hoeks, and R. Mensink, unpublished data). Inclusion criteria were stable statin treatment, age 18–70 y, BMI ≤32 kg/m$^2$, diastolic blood pressure ≤95 mm Hg, and systolic blood pressure ≤200 mm Hg. Individuals with proteinuria, glucosuria, clinical manifestations of hepatic disorders, diabetes mellitus, or active cardiovascular disease within a period of 6 mo preceding the study were excluded. The study was approved by the local Medical Ethics Committee. Prior to enrolment, all participants signed an informed consent form.

*Diets and design.* The present study was a randomized, double-blind, placebo-controlled dietary intervention trial. Participants were asked to replace their usual spread with an experimental "light" margarine (40% fat). During the first 5 wk (run-in period), the participants used a control margarine. Hereafter, the participants were randomly allocated to 1 of 3 experimental groups, stratified for age and sex. The study continued with an 85-wk intervention period, during which the first group continued with the control margarine, the second group with a plant sterol ester- (2.5 g/d) enriched margarine, and the 3rd group with a plant stanol ester- (2.5 g/d) enriched margarine (Table 1). In the margarines, plant sterols [sitosterol (49%), campesterol (31%), and stigmasterol (16%)] (Unilever) and plant stanols [sitostanol (70%) and campestanol (29%)] (Walter Rau Neusser Öl und Fett AG) were esterified with sunflower oil. The 3 spreads, which were similar in appearance, taste, absorbable fat content, and energy content, were numerically coded. The margarines were packed in tubs of 250 g; participants were advised to weigh the tubs daily to ensure a consumption of 30 g/d. Participants were asked not to change their habitual diet, level of physical exercise, smoking habits, or alcohol consumption during the study, and to write down in a diary any signs of illness, change of medication, and the daily amount of margarine used. In wk 5, 50, and 90, the participants returned the used tubs from the previous 8 wk, which were weighed to calculate weekly margarine intake. In addition, individual energy and nutrient intake during the previous 4 wk was recorded using an FFQ (33). Venous blood samples were collected from fasting participants for analysis of serum plant sterol and stanol concentrations at wk 4, 5, 49, 50, 89, and 90. At the end of the run-in period (wk 5) and again after the 85-wk intervention period (wk 90), the participants underwent a cognitive assessment and completed 2 self-report questionnaires.

**TABLE 1** Composition of the control and experimental margarines

| Ingredient | Control margarine | Plant sterol ester margarine | Plant stanol ester margarine |
|---|---|---|---|
| | | *g/100 g margarine* | |
| Protein | 0 | 0 | 0 |
| Carbohydrates | 2.5 | 2.2 | 2.8 |
| Total fat[1] | 35.4 | 35.2 | 35.0 |
| SFA | 7.6 | 8.0 | 8.7 |
| MUFA[3] | 7.5 | 7.4 | 8.7 |
| PUFA | 20.1 | 19.7 | 17.4 |
| Trans fat | 0.4 | 0.6 | 0.5 |
| Total sterol[2] | 0.2 | 7.7 | 8.0 |
| Cholesterol | NA[3] | , 0.1 | , 0.1 |
| Sitosterol | | 3.6 | 0.2 |
| Campesterol | | 2.0 | 0.2 |
| Stigmasterol | | 1.5 | 0 |
| Sitostanol | | , 0.1 | 5.3 |
| Campestanol | | , 0.1 | 2.1 |
| Other plant sterols | | 0.5 | 0 |

[1] Digestible fat, excluding the plant sterols.
[2] Total amount of sterol equivalents added in esterified form.
[3] Abbreviations: MUFA, monounsaturated fatty acid; NA, not analyzed.

*Plant sterol and stanol analysis.* Serum plant sterol (sitosterol and campesterol) and plant stanol (sitostanol and campestanol) concentrations in venous blood were analyzed by GC-MS, as described previously (25). Serum total cholesterol concentrations were analyzed as described by Plat et al. (33). Samples from wk 4 and 5, wk 49 and 50, and wk 89 and 90 were pooled before analysis. All samples from 1 individual were analyzed within the same sequence. Absolute plant sterol and stanol serum concentrations are expressed as μmol/L and cholesterol-standardized serum concentrations as $10^2$ 3 μmol/mmol cholesterol.

*Cognitive test battery.* The Mini-Mental State Examination was used to screen for symptoms of dementia (34). The maximum test score is 30. Individuals with an overall test score ≤ 24, which was considered an indication of an increased risk for dementia, were excluded from statistical analysis.

The Visual Verbal Word Learning Task (WLT)[7] was used to assess learning capacity as well as recall and retrieval from long-term memory (35). In 5 trials, 15 frequently occurring Dutch words were visually presented in a fixed order at 2-s intervals. Total free recall, delayed recall after 20 min, and recognition were measured.

The Stroop Color-Word Interference test was used to test selective attention (36). Participants were required to read aloud color names printed in black (subtask I), name the color of colored patches (subtask II), and name the ink color of color names printed in an incongruous color (subtask III). The outcome parameters were speed on subtask I, speed on subtask II, and speed on subtask III.

[7] Abbreviations used: CST, Concept Shifting test; LDST, Letter-Digit Substitution test; WLT, Visual Verbal Word Learning Task.

The Concept Shifting test (CST) is a test of behavioral planning (37). Participants were asked to cross out 16 items presented on a test sheet as fast as possible in the right order [1–2–3–4 (subtask A), A–B–C–D (subtask B), 1–A–2–B (subtask C)]. The outcome parameters were speed on subtask A, speed on subtask B, and speed on subtask C.

We used the Letter-Digit Substitution test (LDST) to measure efficiency of operations in working memory (38). Participants were asked to replace letters presented on a test sheet by their corresponding digits, as indicated by a key showing 9 numbers paired with different letters. The total number of correct substitutions completed within 90 s was used as a measure of LDST performance.

*Data reduction.* To limit the number of dependent variables and to improve the robustness of the underlying cognitive construct, we clustered the raw test scores into 3 composite performance indices (39). First, the raw test scores were transformed into Z-scores by subtracting the mean score of the pooled measurements (i.e. baseline and 85 wk of follow-up) from the individual test score and dividing this by the mean SD of the pooled measurements [Z = ($x$ – mean)/SD]. Hereafter, the Z-scores were averaged, resulting in the following composite scores: memory [(ZWLTtot + ZWLTdr + ZWLTrec)/3], simple information processing speed [(ZStr1 + ZStr2 + ZCSTa + ZCSTb)/4], and complex information processing speed [(ZStr3 + ZCSTc)/2], where WLTtot is total free recall on the WLT, WLTdr is delayed recall after 20 min on the WLT, WLTrec is recognition on the WLT, Str1-3 are speed on subtasks I-III of the Stroop Color-Word Interference Test, and CSTa–c are speed on subtasks A–C on the CST. The signs of the 2 speed scores were inverted to reflect above normal performance when positive and below normal when negative. For means of comparison, LDST performance was also transformed into a Z-score.

*Questionnaires.* We used the Cognitive Failure Questionnaire to measure the frequency of cognitive failures in everyday life (40,41). A higher overall score (range 0–100) indicated decreased subjective cognitive functioning.

Mood was assessed with the depression subscale of the Symptom Checklist 90 (42). Higher sum scores (range 16–80) represented increased depressed mood.

*Education.* Level of education, assessed by classifying formal schooling according to the Dutch educational system (43), was categorized as either low or average/high (35,36,38).

*Statistical analysis.* Normal probability-probability plots indicated skewness of the depression subscale of the Symptom Checklist 90 scores, which was corrected by log-transformation. Homogeneity of variances was assessed with Levene's test for equality of error variances. Chi-square tests were used to determine whether the experimental groups differed in terms of sex and level of education. We used 1-way ANOVA to assess possible group differences in terms of age and the serum concentrations of the plant sterols and stanols at baseline, as well as the serum concentration of sitosterol at the end of the intervention period. In the case of overall significant group differences, post-hoc Dunnett's $t$ tests were performed to compare the 2 intervention groups with the control group. Because of heterogeneity of variances, group differences in the serum concentrations of campesterol, sitostanol, and campestanol at the end of the intervention period were assessed using the nonparametric Kruskal-Wallis test. We used Mann-Whitney U-tests to compare the 2 intervention groups with the control group. In addition, these nonparametric tests were used to examine group differences in absolute changes in serum plant sterol concentrations from baseline to the end of the intervention period.

To investigate the effects of plant sterol or stanol ester consumption on cognitive performance, subjective cognitive functioning, and self-reported mood, univariate ANCOVA were performed. In each analysis, the baseline test score of the dependent variable was included as a covariate. The between-subjects factor "group" was used to evaluate the overall intervention effect. A power calculation for ANCOVA on the primary outcome measures with a medium effect size of 0.15 (44) revealed a statistical power of 0.79.

Data are presented as means ± SD. Differences were considered significant at $P$ , 0.05. All analyses were performed using SPSS 16.0 for Apple Macintosh.

## Results

*Participants.* Ninety-two individuals showed interest in our study (Fig. 1). After screening, 59 individuals were considered eligible for study entry. Two individuals withdrew before the start of the study. One person in the plant sterol group dropped out in wk 40 because of diagnosis of type 2 diabetes mellitus. Two individuals in the plant stanol group dropped out; 1 in wk 48, because of discontinuation of statin treatment due to side effects, and the other in wk 70, when starting in a weight loss program. In total, 54 individuals (32 men, 22 women) completed the study. The test results of 1 male participant in the plant stanol group were excluded from statistical analyses because of a low Mini-Mental State Examination score ( , 24).

The 3 experimental groups did not differ significantly in terms of age, sex, level of education, and baseline serum concentrations of the plant sterols and stanols (Table 2; Supplemental Table 1). Energy intake, the proportions of energy from macronutrients, cholesterol intake, and BMI did not differ between the 3 groups at baseline and did not change significantly during the study. All individuals performed within the limits for nonpathological neurocognitive functioning (35–38).

Compliance with the trial protocol, as indicated by tub weighing and blood sampling, was excellent. At the end of the intervention period, serum plant sterol concentrations differed significantly between the plant sterol group and the control group, whereas serum plant stanol concentrations differed significantly between the plant stanol group and the control group (Table 2). After 85 wk of supplementation, serum sitosterol and campesterol concentrations were significantly increased in the plant sterol group compared with the control group ($P$ , 0.05 for the difference in absolute changes). In the plant stanol group, serum sitostanol and campestanol concentrations were markedly elevated compared with the control group ($P$ , 0.05 for the difference in absolute changes).

The effects of supplementation with plant sterol or stanol esters on serum cholesterol concentrations in the present study have been described in detail by De Jong et al. (32). In short, compared with the control group, serum total cholesterol concentrations were reduced by 5.1% in the plant sterol group ($P$ = 0.09 for the difference in absolute changes) and 9.4% in the plant stanol group ($P$ , 0.05) after 85 wk of intervention and

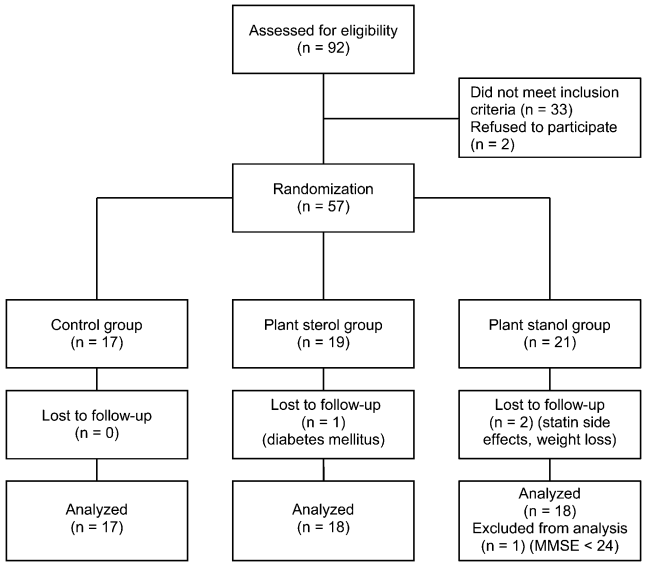


**FIGURE 1** Participant flow diagram of this study.

**TABLE 2** Participants' characteristics at baseline and after 85 wk of supplementation with control margarine, plant sterol ester-enriched margarine, or plant stanol ester-enriched margarine[1]

| | Control | Plant sterol | Plant stanol |
|---|---|---|---|
| *n* | 17 | 18 | 18 |
| Age, *y* | 60.4 ± 7.4 | 59.8 ± 6.2 | 59.0 ± 7.1 |
| Female sex, *n (%)* | 6 (35.3) | 8 (44.4) | 8 (44.4) |
| Low level of education, *n (%)* | 5 (29.4) | 9 (50.0) | 7 (38.9) |
| Sitosterol, *10²* × *μmol/mmol cholesterol* | | | |
| Baseline | 174.4 ± 62.7 | 212.4 ± 109.2 | 164.5 ± 84.8 |
| End of intervention period | 178.1 ± 75.1 | 296.7 ± 122.4* | 136.9 ± 62.4 |
| Campesterol, *10²* × *μmol/mmol cholesterol* | | | |
| Baseline | 265.8 ± 112.3 | 330.7 ± 177.6 | 255.3 ± 141.6 |
| End of intervention period | 242.2 ± 99.8 | 685.6 ± 287.8* | 184.6 ± 72.6 |
| Sitostanol, *10²* × *μmol/mmol cholesterol* | | | |
| Baseline | 2.9 ± 0.8 | 3.2 ± 1.1 | 3.1 ± 1.3 |
| End of intervention period | 2.9 ± 0.8 | 2.6 ± 0.7 | 23.3 ± 16.3* |
| Campestanol, *10²* × *μmol/mmol cholesterol* | | | |
| Baseline | 3.3 ± 0.7 | 3.8 ± 1.3 | 3.5 ± 1.2 |
| End of intervention period | 3.2 ± 0.6 | 3.4 ± 1.0 | 20.5 ± 14.0* |

[1] Values are means ± SD or *n* (%). *Different from control group, $P < 0.05$.

serum LDL-cholesterol concentrations were lowered by 8.7% in the plant sterol group ($P = 0.08$) and 13.1% in the plant stanol group ($P < 0.05$).

*Neurocognitive functioning and self-reported mood.* Plant sterol or stanol ester supplementation did not affect memory, simple information processing speed, complex information processing speed, or LDST performance (Table 3). In addition, long-term administration of plant sterol or stanol esters did not significantly affect subjective cognitive functioning or mood (Table 3).

**TABLE 3** Effects of 85-wk consumption of plant sterol or plant stanol ester-enriched margarines on neurocognitive functioning and mood in hypercholesterolemic individuals on statin treatment[1]

| | Control | Plant sterol | Plant stanol | *P* |
|---|---|---|---|---|
| *n* | 17 | 18 | 18 | |
| Memory[2] | | | | 0.25 |
| Baseline | −0.14 ± 0.54 | −0.06 ± 0.87 | −0.16 ± 0.97 | |
| End of intervention period | −0.06 ± 0.84 | 0.12 ± 0.96 | 0.29 ± 0.77 | |
| Simple speed[2] | | | | 0.24 |
| Baseline | 0.06 ± 0.74 | −0.24 ± 0.58 | 0.27 ± 0.81 | |
| End of intervention period | −0.02 ± 0.85 | −0.22 ± 0.61 | 0.02 ± 1.17 | |
| Complex speed[2] | | | | 0.55 |
| Baseline | −0.05 ± 0.78 | −0.25 ± 1.03 | 0.15 ± 0.95 | |
| End of intervention period | 0.02 ± 0.69 | −0.23 ± 0.63 | 0.23 ± 1.00 | |
| LDST performance[2] | | | | 0.75 |
| Baseline | 0.06 ± 0.93 | −0.20 ± 0.86 | 0.20 ± 1.03 | |
| End of intervention period | −0.05 ± 0.92 | −0.24 ± 1.22 | 0.23 ± 1.02 | |
| Subjective cognitive functioning[3] | | | | 0.64 |
| Baseline | 27.0 ± 11.6 | 33.8 ± 11.2 | 31.6 ± 12.2 | |
| End of intervention period | 27.7 ± 12.2 | 33.0 ± 8.1 | 28.7 ± 9.2 | |
| Mood[3] | | | | 0.53 |
| Baseline | 20.1 ± 3.8 | 23.1 ± 7.3 | 22.7 ± 7.3 | |
| End of intervention period | 19.8 ± 3.7 | 22.5 ± 6.7 | 20.8 ± 5.3 | |

[1] Values are means ± SD.
[2] Cognitive performance indices and LDST performance are expressed as Z-scores.
[3] Subjective cognitive functioning and mood are expressed as overall scores on the Cognitive Failure Questionnaire and the depression subscale of the Symptom Checklist 90, respectively.

## Discussion

In this study, we showed for the first time, to our knowledge, that long-term administration of plant sterol and stanol esters did not have any effects on cognitive performance, subjective cognitive functioning, or self-reported mood in statin-treated hypercholesterolemic individuals, despite a pronounced increase in the serum concentrations of these compounds.

Consistent with the present findings, there have not been any reports suggesting abnormal neurocognitive functioning in sitosterolemic patients, who have genetically determined high concentrations of plant sterols and stanols in serum and tissues (45,46), although mouse models have indicated that sitosterolemia may be accompanied by elevated brain levels of plant sterols and stanols (26). It should be noted, however, that extensive research on cognitive performance in sitosterolemic individuals appears to be lacking.

The absence of any effects of plant sterols and stanols on cognitive performance and mood suggests that, in contrast with peripheral cellular membranes, these compounds may not be incorporated into neuronal membranes, thereby leaving membrane fluidity and signal transduction processes in the brain unaffected. In addition, the present results suggest that the cholesterol-lowering properties of plant sterols and stanols may not affect brain cholesterol levels, which is supported by animal studies (22,23) and the finding that serum concentrations of 24 (S)-hydroxycholesterol, a surrogate marker for brain cholesterol homeostasis (47), remained unchanged over the 85-wk intervention period (32).

Some methodological aspects of our study deserve to be addressed to put the present results in perspective. One of the strengths of the present study is the 85-wk duration of the intervention period, which enabled us to investigate the effects of genuine long-term supplementation with plant sterols and stanols, closely resembling the conditions of administration as adjunctive cholesterol-lowering treatment. In addition, we assigned our participants to separate experimental plant sterol and stanol groups, which allowed the neurocognitive effects of plant sterols and stanols to be compared.

Second, from the significant changes in the serum concentrations of plant sterols and stanols in the 2 intervention groups compared with the control group, it may be concluded that the intervention was successful. Although it has not yet been evaluated in humans, we inferred from animal studies that dietary supplementation with plant sterols and stanols would increase the brain levels of these compounds (20,22,23). Third, the cognitive test battery used in the present study has been proven a sensitive tool for assessing neurocognitive functioning (48,49). The use of composite scores for individual performance on the various cognitive domains, which improves the robustness of the underlying cognitive construct (39), particularly facilitated the detection of possible changes in cognitive performance due to supplementation with plant sterol or stanol esters (50). The data distribution of the cognitive performance indices in the present study is comparable to that reported in other population-based studies using a Z-transformation of cognitive test scores (49,51).

Fourth, the statistical power (i.e. 0.79) was deemed sufficiently large to detect possible effects of the intervention on

cognitive performance and mood. Nevertheless, the present sample size did not allow for the detection of very small effects of the intervention and therefore we cannot rule out completely that plant sterols and stanols might exert some minor effects on neurocognitive functioning.

Finally, it should be noted that the present results were obtained in a study sample of hypercholesterolemic individuals on stable statin treatment. The various statins used by the participants in the present study have been reported by De Jong et al. (32). About one-half of the participants in each experimental group used lipophilic statins, which may enter the brain. It is unlikely, however, that the use of statins may have influenced the present results. In the first place, statin treatment does not seem to alter brain cholesterol metabolism in hypercholesterolemic patients (52). In addition, although rare instances of statin-associated memory loss have been described (53), animal studies have indicated that the concentrations needed for memory impairment are generally much higher than the dose normally used in cholesterol-lowering therapy (31). Furthermore, various clinical trials have reported no significant effects of statins on cognitive performance (54–57). Finally, it is worth noting that statins tend to increase serum plant sterol and stanol levels (58), which makes individuals on statin treatment an excellent population to study the effects of plant sterols and stanols.

The main benefit of including only hypercholesterolemic persons in the present study is that it increased the probability of detecting putative effects of plant sterols and stanols on neurocognitive functioning by reducing statistical variance among the participants. A disadvantage of the concomitant increase in the study's internal validity, however, is a reduction in its external validity. Therefore, the present findings cannot simply be generalized to the general population. Clearly, although hypercholesterolemic patients on statin treatment are a target population for plant sterol and stanol supplementation, future studies should also investigate the suitability of long-term use of plant sterols and stanols in healthy individuals and sitosterolemic patients, particularly because dietary supplementation with plant stanols in sitosterolemic individuals may result in prolonged retention of these compounds in plasma (59).

In conclusion, long-term use of plant sterols and stanols at recommended intakes of 2.5 g/d, which was already regarded safe from a physiological perspective, does not appear to exert any effects on the level of neurocognitive functioning and mood in humans. Nevertheless, further research is warranted to elucidate the exact fate and possible effects of plant sterols and stanols in the human brain.

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