Food and Chemical Toxicology Volume 44, Issue 10 , October 2006, Pages 1682-1688

doi:10.1016/j.fct.2006.05.006

Effectiveness of customary use of phytosterol/-stanol enriched margarines on blood cholesterol lowering

Marion Wolfs[a], Nynke de Jong[*, a], Marga C. Ocké[a], Hans Verhagen[a] and W.M. Monique Verschuren[a]

[a]National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands

[*]Corresponding author. Tel.: +31 30 274135; fax: +31 30 2744466.

Abstract

Postlaunch monitoring of functional foods can encompass monitoring of effectiveness under conditions of customary use. To this end, the effectiveness of phytosterol/-stanol enriched margarine consumption in free-living conditions was investigated with data from the Dutch "Doetinchem cohort study". In total, 4505 subjects (aged 26–70 years) were examined in 1994–1998 and re-examined during 1999–2003. A general and a food frequency questionnaire and non-fasting blood samples for total and HDL cholesterol determination were obtained. Subjects were stratified into phytosterol/-stanol enriched margarine users (n = 84) and non-users (n = 4421) based on the re-examination data, as these margarines were available on the Dutch market from 1999 onwards.

Mean spontaneous daily use (g ± SD) of phytosterol-containing margarine (n = 71) was 15 ± 8 and of phytostanol-containing margarine (n = 13) 9 ± 6. After five years, total blood cholesterol had increased with 0.26 mmol/l in non-users while it had not significantly changed in users. The difference in total blood cholesterol change in users versus non-users was −0.30 mmol/l ($p < 0.001$). The beneficial effect of the phytosterol/-stanol enriched margarine, used under customary conditions can be characterized as a stabilization of cholesterol levels. This is the first report finding a modest beneficial effect on blood cholesterol level under customary conditions thereby partly confirming findings from clinical trials.

1. Introduction

To our opinion a postlaunch monitoring (PLM) system should not only notify, analyse and evaluate potential side effects of a specific food, and investigate consumption patterns, but should also perform a risk-benefit analyses in which both the positive and negative health effects are weighed on a population level. In order to be able to do so population data on effectiveness of foods rather than efficacy data obtained in controlled clinical settings are required in addition to customary intake data and data on (un)known adverse effects.

Serum total cholesterol is a major risk factor for coronary heart diseases. A meta-analysis of Law et al. (1994) showed that a long-term reduction in serum total cholesterol of 10% lowers the risk of ischemic heart disease by 50% at the age of 40 and by 20% at the age of 70. Thus, cholesterol-lowering strategies could result in a substantial health benefit at a population level. In many clinical trials, it has been reported that phytosterols or -stanols, when consumed over 1 g per day, induce a significant reduction of serum total cholesterol and low-density lipoprotein (LDL) cholesterol concentration (Law, 2000, Miettinen et al., 1995, Weststrate and Meijer, 1998, Hendriks et al., 1999 and Hallikainen et al., 2000). Dosages of 1.5–3 g phytosterols and -stanols per day were shown to reduce total serum cholesterol by 8–17% and LDL cholesterol by 9–19% (Amundsen et al., 2002), whereas high-density lipoprotein (HDL) cholesterol concentrations remained unaffected. These effects have been confirmed in a meta-analysis of 41 trials in which even an additive effect on cholesterol-lowering drug use was demonstrated (Katan et al., 2003). Without adherence to a controlled diet, the consumption of 20 g phytosterol/-stanol enriched margarine has been shown to have

a lipid lowering effect (Hendriks et al., 2003 and Geelen et al., 2002) and to augment the LDL cholesterol-lowering effect of other cholesterol-lowering strategies (Nestel et al., 2001). These results have lead to the market introduction of several phytosterol/-stanol enriched foods. The level of evidence of the use of these foods on blood cholesterol lowering is sufficient to meet the criteria laid down in the Passclaim consensus report (Aggett et al., 2005). As the foods enriched with phytosterol esters are regarded as novel foods extensive safety evaluations have been performed prior to market launch, and these have all been published in this journal (Lea et al., 2004, Baker et al., 1999, Wolfreys and Hepburn, 2002, Sanders et al., 2000, Ayesh et al., 1999, Weststrate et al., 1999, Waalkens-Berendsen et al., 1999 and Hepburn et al., 1999). Although phytostanol esters were not subject to the novel foods regulation in Europe for formal reasons, also these have gone through safety testing procedures (Slesinski et al., 1999, Turnbull et al., 1999a, Turnbull et al., 1999b, Turnbull et al., 1999c and Katan et al., 2003). Also some PLM investigations have been done upon request by the EU (SCF, 2002b and SCF, 2002a). The main focus of these PLM investigations were on consumption levels, consumption patterns by target and/or non-target groups, and on consumer notifications of side effects. Until now the effects of phytosterol/-stanol enriched foods have only been studied with controlled intake of phytosterols or -stanols. To our knowledge, no study has been published in which the effect of foods enriched with phytosterols/-stanols on blood lipids has been investigated under free-living conditions without a controlled diet. For PLM purposes we are interested in the health benefits of the consumption of these type of foods in the general target population. We, therefore, investigated the consumption level of phytosterol/-stanol enriched margarines under free-living conditions, and the subsequent effect on blood cholesterol levels.

2. Materials and methods

Data were available from the Dutch Doetinchem cohort study for 4505 subjects (2101 males, 2320 females, aged 26–70 years) examined in the years 1994–1998 and re-examined at five years follow-up during 1999–2003. The main objective of this study is to investigate changes in time of risk factors and way of live within the same individuals. Furthermore the relationship between changes in way of live and changes in risk factors is studied. Details of this study have been published elsewhere (Blokstra et al., 2006). A general questionnaire and a validated food frequency questionnaire asking for usual intake in the previous year were filled out (Ocké et al., 1997). Participants were able to tick multiple types of bread spreads. For each indicated type of bread spread, one or two brandnames of the breadspread used could be filled out in an open section (e.g. phytosterol-enriched margarine: Becel pro.activ®; phytostanol-enriched margarine: Benecol®). The amount of breadspread 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. At least until 2003 phytosterol/-stanol enriched margarines were the main sources of these substances on the Dutch market. Other phytosterol/-stanol enriched foods have only been introduced from 2003 onwards. Non-fasting blood samples for total and HDL cholesterol determination were obtained using a standardised protocol (Houterman et al., 2001).

Subjects were stratified into phytosterol/-stanol enriched margarine users ($n = 84$) and non-users ($n = 4421$) based on the follow-up data, as these margarines were available on the Dutch market from 1999 onwards. For descriptive purposes absolute numbers and percentages of general characteristics of the enriched margarine users and non-users were calculated. Some of the general characteristics are presented as mean ± SD. Statistical differences between the general characteristics of users vs non-users were tested with χ^2-test or a Student's t test.

In order to be able to describe the enriched margarine users more thoroughly and to compare with cholesterol-lowering medicine users we have divided the population in four groups for further analysing: i.e. phytosterol/-stanol enriched margarine users (subjects who used enriched margarine but no cholesterol-lowering drugs, $n = 72$), cholesterol lowering drug users (subjects who used cholesterol-lowering drugs but no enriched margarine, $n = 218$), combination users (subjects who used both enriched margarine and cholesterol-lowering drugs, $n = 12$) and total non-users (subjects who used neither enriched margarine or

cholesterol-lowering drugs, $n = 4205$). We compared the general characteristics of these four groups, but we only present those characteristics that are statistically different. We do not have information on the type of cholesterol-lowering drugs that were used, but in accordance to the major trend in affluent countries an increasing majority will use statins.

Because producers of the phytosterol/-stanol enriched margarines claim that about a 10% decline in total blood cholesterol concentration can be achieved by the recommended consumption level of these products, we calculated the percentage of subjects that attained a decline in total blood cholesterol level of ≥10%. We also calculated the average amount of the enriched margarine consumed daily among the users, and how many of the users took the recommended amount of 20 g of margarine per day.

Effectiveness of usage was analysed through calculating the difference in total blood cholesterol change between baseline and re-examination in the four groups. Similarly, we calculated the change in HDL cholesterol and the total/HDL cholesterol ratio. These results are presented as mean ± SD. The difference in each group was tested with a paired *t*-test and between groups with ANOVA. When a significant difference between groups was detected, a Bonferroni test was performed to test which differences were significant while adjusting for multiple comparisons.

Multiple regression analysis was used to analyze the effect of phytosterol/-stanol enriched margarine on the observed lipid changes between baseline and re-examination, while variables known to influence changes in blood lipid concentrations such as sex, age, alcohol intake, fat intake, BMI, smoking behavior, diabetes, the use of cholesterol lowering drugs and an interaction term for use of enriched margarine and cholesterol-lowering drugs, were entered into the model as covariates. The mean total blood cholesterol ((total blood cholesterol at re-examination + baseline total blood cholesterol)/2) was put into the model to correct for regression to the mean. To compare the quantitative blood cholesterol change of the enriched margarine with that of blood cholesterol-lowering drugs, we also analysed the effect of those drugs by multiple regression analysis. The crude as well as the adjusted results are presented (ß (95% CI)).

For all the analyses a significance level of 0.05 was chosen. The data analyses were performed using the statistical software package SAS version 9.1.

3. Results

No differences were observed between the enriched margarine users and non-users for most general characteristics, except for alcohol use, dietary fat intake, baseline total blood cholesterol, prevalence of ever being diagnosed with high cholesterol level and cholesterol-lowering drug use (Table 1). There were more subjects who drank more than two glasses of alcohol per day, among enriched margarine users compared to non-users (30% vs. 18%), and among the users there were less subjects who had a fat intake greater than 30 en% (74% vs. 89%). Users of enriched margarine had a higher baseline total blood cholesterol level than non-users (6.2 vs. 5.5 mmol/l). Similarly, among the users there where more subjects ever diagnosed with high cholesterol level compared to non-users (56% vs. 17%) and more users of enriched margarine used cholesterol-lowering drugs. There were no statistically significant differences ($p > 0.05$) between men and women for baseline values of total serum cholesterol (data not shown).

Food and Chemical Toxicology Volume 44, Issue 10 , October 2006, Pages 1682-1688

Table 1.

General characteristics of users versus non-users of phytosterol/-stanol enriched margarine in a Dutch cohort (*n* = 4505), 1999–2003

Characteristic	**Users**		**Non-users**[a]		***p*-Value**[b]
	***n* = 84**	**%**	***n* = 4421**	**%**	
Gender					
Male	37	44	2101	47	ns
Female	47	56	2320	53	
Age, yrs (mean ± SD)	53.7 ± 8.5		51.2 ± 10.2		ns
Education					
Low	37	44	2210	50	ns
Middle	20	24	1238	28	
High	27	32	973	22	
Subjective health					
Bad	1	1	43	1	ns
Moderate	9	11	574	13	
Good	74	88	3804	86	
Smoking					
Never	28	34	1546	35	ns
Ex	43	52	1727	39	
Current	12	14	1148	26	
Alcohol use					
None	22	26	1501	34	0.018
1–2 glasses/d	37	44	2126	48	
>2 glasses/d	25	30	794	18	
BMI, kg/m^2 (mean ± SD)	26.6 ± 4.1		26.3 ± 4.0		ns
Blood pressure, Hg mm (mean ± SD)					
Systolic	133 ± 18		130 ± 18		ns
Diastolic	87 ± 13		82 ± 11		ns
Ever diagnosed with high cholesterol level					
Yes	47	56	764	17	<0.0001
No	31	37	3296	75	
Do not know	6	7	361	8	
Cholesterol-lowering drug usage					
Yes	12	14	218	5	<0.0001
No	72	86	4205	95	
Diabetes					
Yes	5[c]	6	107	2	ns
No	78	94	4314	98	
Stroke or MI					
Yes	4	5	152	3	ns
No	80	95	4269	97	
Dietary fat intake (en% fat)					
≤30	22	26	508	11	<0.0001
>30	62	74	3913	89	
Activity pattern[d]					
Low	35	42	1839	42	ns
High	49	58	2582	58	
Baseline total blood cholesterol, mmol/l (mean ± SD)[e]	6.2 ± 1.0		5.5 ± 1.0		<0.0001

[a] Numbers vary due to missing values.
[b] χ^2-test or *t*-test.
[c] Of which two persons reported to have type I diabetes.
[d] Low = less than 3.5 h/week moderate or strenuous activity, high = more than 3.5 h/week moderate or strenuous activity.
[e] Values obtained from 1994 to 1998.

When we compared the four groups: i.e. enriched margarine users, cholesterol-lowering drug users, combination users and non-users, it appeared that a high fat intake was less prevalent among the combination users (50% ate more than 30 en% fat), compared to the total non-users (Table 2). In the combination group and the cholesterol-lowering drug using group, all subjects were ever diagnosed with a high cholesterol level. Among the enriched margarine users this was the case for 49%, and among the total non-using group 13% was ever diagnosed with high cholesterol level.

Table 2.

Characteristics of phytosterol/-stanol enriched margarine users, cholesterol-lowering drug users, combination users and non-users, in a Dutch cohort (*n* = 4505)[a]

	Enriched margarine users (*n* = 72) *n* (%)	**Cholesterol-lowering drug users (*n* = 218) *n* (%)**	**Combination users (*n* = 12) *n* (%)**	**Non-users (*n* = 4203) *n* (%)**
Age, yrs (mean ± SD)	53.6 ± 8.8[a]	59.5 ± 8.1[b]	53.9 ± 6.8	50.8 ± 10.2[a]
Ever diagnosed with high cholesterol level	35 (49%)[a]	218 (100%)[b]	12 (100%)[b]	546 (13%)[c]
Stroke or MI				
Yes	0 (0%)[a]	68 (31%)[b]	4 (33%)[b]	84 (2%)[a]
No	72 (100%)	150 (69%)	8 (67%)	4119 (98%)
Dietary fat intake (en% fat)				
≤30	16 (22%)[a]	46 (21%)[a]	6 (50%)[b]	462 (11%)[c]
>30	56 (78%)	172 (79%)	6 (50%)	3741 (89%)

Only those values that are significantly different are presented.

Different superscripts (a, b or c) represent statistically significantly differences ($p < 0.05$) among the groups.

The non-using group had a significantly lower baseline total blood cholesterol level compared to the other groups (Table 3). Mean spontaneous daily use for phytosterol-eniched margarine was 15 ± 8 g, ranging from 0.6 to 38 g (*n* = 71), and for phyostanol-enriched margarine was 9 ± 6 g, ranging from 0.5 to 21 g (*n* = 13). Of the 84 users, 81% ate less than the recommended 20 g/d. Men used significantly more of the enriched margarine than women (16 ± 9 g/d vs. 12 ± 7 g/d, $p = 0.01$). Between the group of enriched margarine users and the combination users there was no significant difference in the mean consumption levels of margarine (14 ± 8 g/d vs. 14 ± 9 g/d).

Table 3.

Baseline data and change in levels upon re-examination of total blood cholesterol, HDL cholesterol, and total blood cholesterol/HDL ratio in phytosterol/-stanol enriched margarine users, cholesterol lowering drug users, combination users and non-users in a Dutch cohort (n = 4505)[a]

	Enriched margarine users (n = 72) mean ± SD	**Cholesterol-lowering drug users (n = 218) mean ± SD**	**Combination users (n = 12) mean ± SD**	**Non-users (n = 4203) mean ± SD**
Total blood cholesterol (mmol/l)				
Baseline	6.25 ± 0.97	6.51 ± 1.35	6.07 ± 0.90	5.44 ± 0.98[a]
5 yrs difference	−0.08 ± 0.83[a]	−1.17 ± 1.34[b]	−1.34 ± 0.78[b]	0.26 ± 0.71[c]
Change ≥ −10% (% of group)	26%[a]	64%[b]	83%[b]	10%[c]
HDL cholesterol (mmol/l)				
Baseline	1.41 ± 0.38[a]	1.19 ± 0.30[b]	1.17 ± 0.24[a,b]	1.39 ± 0.38[a]
5 yrs difference	−0.00 ± 0.18	0.03 ± 0.21	0.05 ± 0.26	−0.01 ± 0.23
Total/HDL ratio (mmol/l)				
Baseline	4.8 ± 1.63[a]	5.8 ± 1.91[b]	5.31 ± 0.95[a,b,c]	4.20 ± 1.48[c]
5 yrs difference	−0.09 ± 1.03[a]	−1.15 ± 1. 67[b]	−1.15 ± 0.91[b]	0.26 ± 1.03[c]

Different superscripts (a, b or c) represent statistically significantly differences (p < 0.05) among the groups.

At re-examination, i.e. during the period of consumption of margarines enriched with phytosterols and phytostanols, total blood cholesterol in the enriched margarine users had on average decreased with 0.08 mmol/l, although this decrease was not significant (p = 0.41). In contrast, total blood cholesterol had significantly (p < 0.0001) increased with 0.26 mmol/l in the total group of non-users. The cholesterol-lowering drug users and combination users had a significant (p < 0.0001) decrease in total blood cholesterol of 1.17 mmol/l and 1.34 mmol/l respectively. A similar effect was found for the total blood cholesterol/HDL ratio. In Table 3, the overall results of the statistical tests among the four groups are presented, whereas in Fig. 1, results are visualised for the change in total blood cholesterol over time.

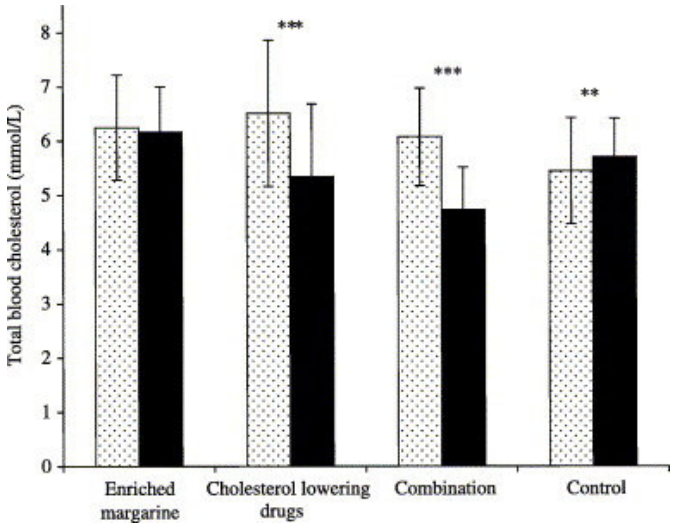

Fig. 1. Baseline data (▒) and data upon re-examination (■) of total blood cholesterol levels in phytosterol/-stanol enriched margarine users, cholesterol-lowering drug users, combination users and non-users in a Dutch cohort (n = 4505). (Statistically significant over time: **p < 0.05, ***p < 0.001.)

There was no significant change in blood HDL cholesterol concentration in each of the four groups. Between men and women, there was no significant difference in five years change in blood cholesterol level ($p > 0.05$).

We calculated the amount of subjects that attained a decline in total blood cholesterol level of ≥10%, because this level of decline is claimed by the producers of the phytosterol/-stanol enriched margarines. Of the enriched margarine users 26% achieved this. For the cholesterol lowering drug users and combination users this was 64% and 83% respectively. Nonetheless, in the non-using group there were also 10% of the subjects who showed a 'spontaneous' decline of ≥10% in total blood cholesterol level (Table 3).

To further determine the effect of phytosterol/-stanol enriched margarine on the observed lipid changes between baseline and re-examination, gender, age, alcohol intake, fat intake, BMI, smoking, diabetes and the use of cholesterol-lowering drugs were entered into a multivariate model as covariates. Of these covariates, cholesterol lowering drug use, age, fat intake and BMI *contributed* significantly to the model. It made no difference whether we put total fat intake or saturated fat intake into the model (results not shown). After correction for the covariates and regression to the mean, the use of enriched margarine had a significant lowering effect on the five years change in total blood cholesterol level of –0.30 mmol/l compared to non-use of enriched margarine (Table 4).

Table 4.

The effect of phytosterol or -stanol enriched margarine use vs. non-use, and the effect of cholesterol-lowering drug use vs. non-use on baseline vs. re-examination in total blood cholesterol and HDL levels, and total blood cholesterol/HDL ratio, according to multivariate regression analysis

	Crude		**Adjusted**	
	ß (95% CI)	***p*-Value**	**ß (95% CI)**	***p*-Value**
Total blood cholesterol 5 yrs difference (mmol/l)				
Enriched margarine	–0.45 (–0.27; –0.63)	<0.0001	–0.30 (–0.14; –0.47)[a]	0.0003
cholesterol-lowering drugs	–1.43 (–1.22; –1.53)	<0.0001	–1.35 (–1.25; –1.45)[b]	<0.0001
HDL cholesterol 5 yrs difference (mmol/l)				
Enriched margarine	0.02 (0.07; –0.03)	0.50	0.01 (0.06; –0.04)[a]	0.66
cholesterol-lowering drugs	0.04 (0.07; 0.01)	0.007	0.03 (0.06; 0.00) [b]	0.04
Total/HDL ratio 5 yrs difference (mmol/l)				
Enriched margarine	–0.43 (–0.19; –0.68)	0.0004	–0.29 -(0.06; –0.52)[a]	0.02
cholesterol-lowering drugs	–1.40 (–1.26; –1.55)	<0.0001	–1.31 (–1.17; –1.46)[b]	<0.0001

[a] Adjusted for age, cholesterol-lowering drug use, fat intake and BMI.
[b] Adjusted for age, phytosterol/-stanol enriched margarine use, fat intake and BMI.

4. Discussion

Blood cholesterol levels remained stable over a period of five years in users of phytosterol and -stanol enriched margarines whereas it increased in non-users. After correction for covariates and regression to the mean, the mean difference in total blood cholesterol levels between users and non-users of the enriched margarine was 0.30 mmol/l (95% CI: 0.14; 0.47).

The observed increase of 0.26 mmol/l in total blood cholesterol levels of non-users can *at least be* explained *partly* by an aging effect as people have turned five years older upon re-examination. It seems therefore that the longer term beneficial health effect of the enriched margarine, used under free-living conditions can be ascribed to a stabilization of the cholesterol level over a longer time period, in contrast with an increase of about 5% in non-

users. Another potential reason for the increase observed in the total cholesterol levels in the non-using group may be an increase in BMI: i.e. 0.7 kg/m^2 *over five years time.*

The reported average daily consumption of 14 g enriched margarine under free-living conditions is lower than the amount of 20 g per day recommended by the producers of the enriched margarines. Only one in five users of the phytosterol or -stanol enriched margarines ate the recommended amount of 20 g of margarine per day or more. Also in a Finnish population it was shown that the use of enriched margarine is often inconsistent (Luoto et al., 2004). This limited consumed amount may have diminished the blood cholesterol-lowering effect of the phytosterol or -stanol enriched margarine. But, given the low average intake of approximately 1.3 g plant sterols per day (≈14 g margarine), the eventual size of the beneficial effect on total blood cholesterol is comparable to what has been found in clinical settings. For example, Hendriks et al. (2003) found a decrease in total blood cholesterol of 0.31 mmol/l (95% CI: 0.20; 0.41) over a one year period, with a relatively low intake of plant sterols of 1.6 g/d.

The free-living conditions of this study have the advantage that results are a representation of what is happening in daily practice and therefore relevant for public health. The disadvantage is that confounding by other factors cannot be excluded. For instance, there was a difference in knowledge about high cholesterol level between users and non-users (56% vs. 18%) and a difference in the amount of en% fat intake. Users of the enriched margarine, especially the combination users ate considerably less fat than non-users. This could indicate that the blood cholesterol level of the users had more declined compared to that of non-users, because users of the enriched margarine act upon the knowledge of having an elevated cholesterol level. This implicates that we might slightly overestimate the sole effect of phytosterol/-stanol enriched margarine consumption and thus present the maximum achieved effect. Although we adjusted for the intake of fat and other factors that influence blood cholesterol, we can not exclude some persisting residual confounding.

In our study population, cholesterol-lowering drug use was more important than the use of margarine enriched with plant sterols/-stanols for a decrease in blood cholesterol level. In total 26% of the enriched margarine users achieved the intended result of a 10% decrease in total blood cholesterol concentration, while this was the case for 64% of those subjects who used cholesterol-lowering drugs. In this study, 12 subjects used both enriched margarine and cholesterol-lowering drugs. A combination of cholesterol-lowering drug therapy with a phytosterol regimen has been the topic of recent intervention trials. Most studies found an additive effect of phytosterols and -stanols on cholesterol lowering drugs (Blair et al., 2000, Simons, 2002 and Katan et al., 2003), but not all (Neil et al., 2001). Although our results showed a trend towards an additive effect, the group of subjects that used both enriched margarine and cholesterol-lowering drugs was too small (n = 12) to have enough power to demonstrate a significant effect. In contrast to this potential beneficial result of a combination therapy there has also been some concern about a potential atherosclerotic and thus negative effect of phytosterol consumption especially when combined with statins (Patel and Thompson, 2006) Further research should elaborate on this issue. As we wrote in the introduction PLM should report on effectiveness of foods in addition to (un)known adverse effects. This paper focused on effectiveness but did not investigate any side effects of phytosterol/-stanol enriched margarine use. Unfortunately, we did not have sufficient power and sufficiently tailored data to perform a complete PLM. Data linkage will be necessary for doing so.

In free-living conditions, the amounts consumed is lower than the recommended intake. Subsequently, the net effect of the phytosterol and -stanol enriched margarine is less than in most clinical trials. At this level of use, over a five years period, the maximum effect achieved was a stabilisation of the cholesterol level rather than the slight increase that is usually seen with aging. Although this effect seems to be modest, it can still reduce the risk of coronary heart disease and thereby result in health benefits in the general population.

To our knowledge, this study is the first to present achieved effects of *customary* phytosterol and -stanol enriched margarine use. As such this confirms to the extensive database on

efficacy of these in controlled clinical trials. This aspect of post launch monitoring is an important addition to the other aspects of post launch monitoring (notification, analysis and evaluation of potential side effects of a specific product, and investigation of consumption patterns) and could be extended to other functional foods and functional food ingredients.

Acknowledgements

The authors would like to thank Dr. Hendriek Boshuizen for statistical advice.

The Doetinchem Cohort Studie was financially supported by the Ministry of Public Health, Welfare and Sports of the Netherlands and the National Institute of Public Health and the Environment, Bilthoven. We thank the epidemiologists and field workers of the Municipal Health Service in Doetinchem for their contribution to the data collection for this study. Project Leader was Dr. W.M.M. Verschuren, datamanagement was performed by A. Blokstra M.Sc, P.E. Steinberger M.Sc and A.W.D. van Kessel M.Sc, logistic support was provided by J. Steenbrink-van Woerden M.Sc. and P. Vissink and secretarial assistance was provided by E. van der Wolf.

We acknowledge the Netherlands Organisation for Health Research and Development (ZonMW) for supporting M. Wolfs (Grant Number 014-12-010).

References

Aggett et al., 2005 P.J. Aggett, J.M. Antoine, N.G. Asp, F. Bellisle, L. Contor, J.H. Cummings, J. Howlett, D.J. Muller, C. Persin, L.T. Pijls, G. Rechkemmer, S. Tuijtelaars and H. Verhagen, PASSCLAIM: consensus on criteria, Eur. J. Nutr. 44 (2005) (Suppl. 1), pp. i5–i30. Full Text via CrossRef

Amundsen et al., 2002 A.L. Amundsen, L. Ose, M.S. Nenseter and F.Y. Ntanios, Plant sterol ester-enriched spread lowers plasma total and LDL cholesterol in children with familial hypercholesterolemia, Am. J. Clin. Nutr. 76 (2002), pp. 338–344. Abstract + References in Scopus | Cited By in Scopus

Ayesh et al., 1999 R. Ayesh, J.A. Weststrate, P.N. Drewitt and P.A. Hepburn, Safety evaluation of phytosterol esters. Part 5. Faecal short-chain fatty acid and microflora content, faecal bacterial enzyme activity and serum female sex hormones in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine, Food Chem. Toxicol. 37 (1999), pp. 1127–1138. SummaryPlus | Full Text + Links | PDF (155 K) | Abstract + References in Scopus | Cited By in Scopus

Baker et al., 1999 V.A. Baker, P.A. Hepburn, S.J. Kennedy, P.A. Jones, L.J. Lea, J.P. Sumpter and J. Ashby, Safety evaluation of phytosterol esters. Part 1. Assessment of oestrogenicity using a combination of in vivo and in vitro assays, Food Chem. Toxicol. 37 (1999), pp. 13–22. SummaryPlus | Full Text + Links | PDF (761 K) | Abstract + References in Scopus | Cited By in Scopus

Blair et al., 2000 S.N. Blair, D.M. Capuzzi, S.O. Gottlieb, T. Nguyen, J.M. Morgan and N.B. Cater, Incremental reduction of serum total cholesterol and low-density lipoprotein cholesterol with the addition of plant stanol ester-containing spread to statin therapy, Am. J. Cardiol. 86 (2000), pp. 46–52. SummaryPlus | Full Text + Links | PDF (183 K) | Abstract + References in Scopus | Cited By in Scopus

Blokstra et al., 2006 Blokstra, A., Smit, H.A., Verschuren, W.M.M. (2006). Changes in lifestyle factors and risk factors for chronic diseases with ageing: The Doetinchem Study 1987–2002. RIVM reportnr 260401003 (in Dutch).

Geelen et al., 2002 A. Geelen, P.L. Zock, J.H. de Vries and M.B. Katan, Apolipoprotein E polymorphism and serum lipid response to plant sterols in humans, Eur. J. Clin. Invest. 32 (2002), pp. 738–742. Full Text via CrossRef | Abstract + References in Scopus | Cited By in Scopus

Hallikainen et al., 2000 M.A. Hallikainen, E.S. Sarkkinen, H. Gylling, A.T. Erkkila and M.I. Uusitupa, 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. 54 (2000), pp. 715–725. Full Text via CrossRef | Abstract + References in Scopus | Cited By in Scopus

Hendriks et al., 1999 H.F. Hendriks, J.A. Weststrate, T. van Vliet and G.W. Meijer, 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. 53 (1999), pp. 319–327. Abstract + References in Scopus | Cited By in Scopus

Hendriks et al., 2003 H.F. Hendriks, E.J. Brink, G.W. Meijer, H.M. Princen and F.Y. Ntanios, Safety of long-term consumption of plant sterol esters-enriched spread, Eur. J. Clin. Nutr. 57 (2003), pp. 681–692. Full Text via CrossRef | Abstract + References in Scopus | Cited By in Scopus

Hepburn et al., 1999 P.A. Hepburn, S.A. Horner and M. Smith, Safety evaluation of phytosterol esters. Part 2. Subchronic 90-day oral toxicity study on phytosterol esters—a novel functional food, Food Chem. Toxicol. 37 (1999), pp. 521–532. SummaryPlus | Full Text + Links | PDF (158 K) | Abstract + References in Scopus | Cited By in Scopus

Houterman et al., 2001 S. Houterman, W.M. Verschuren, C.M. Oomen, C.M. Boersma-Cobbaert and D. Kromhout, Trends in total and high-density lipoprotein cholesterol and their determinants in The Netherlands between 1993 and 1997, Int. J. Epidemiol. 30 (2001), pp. 1063–1070. Full Text via CrossRef | Abstract + References in Scopus | Cited By in Scopus

Katan et al., 2003 M.B. Katan, S.M. Grundy, P. Jones, M. Law, T. Miettinen and R. Paoletti, Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels, Mayo Clin. Proc. 78 (2003), pp. 965–978. Abstract + References in Scopus | Cited By in Scopus

Law, 2000 M.R. Law, Plant sterol and stanol margarines and health, BMJ 320 (2000), pp. 861–864. Full Text via CrossRef

Law et al., 1994 M.R. Law, N.J. Wald and S.G. Thompson, By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease?, BMJ 308 (1994), pp. 367–372. Abstract + References in Scopus | Cited By in Scopus

Lea et al., 2004 L.J. Lea, P.A. Hepburn, A.M. Wolfreys and P. Baldrick, Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides, Food Chem. Toxicol. 42 (2004), pp. 771–783. SummaryPlus | Full Text + Links | PDF (488 K) | Abstract + References in Scopus | Cited By in Scopus

Luoto et al., 2004 R. Luoto, M. Simojoki, A. Uutela, J.D. Boice Jr., J.K. McLaughlin and P. Puska, Consistency of use of plant stanol ester margarine in Finland, Public Health Nutr. 7 (2004), pp. 63–68.

Miettinen et al., 1995 T.A. Miettinen, P. Puska, H. Gylling, H. Vanhanen and E. Vartiainen, Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population, N. Engl. J. Med. 333 (1995), pp. 1308–1312. Full Text via CrossRef | Abstract + References in Scopus | Cited By in Scopus

Neil et al., 2001 H.A. Neil, G.W. Meijer and L.S. Roe, Randomised controlled trial of use by hypercholesterolaemic patients of a vegetable oil sterol-enriched fat spread, Atherosclerosis 156 (2001), pp. 329–337. SummaryPlus | Full Text + Links | PDF (125 K) | Abstract + References in Scopus | Cited By in Scopus

Nestel et al., 2001 P. Nestel, M. Cehun, S. Pomeroy, M. Abbey and G. Weldon, Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods, Eur. J. Clin. Nutr. 55 (2001), pp. 1084–1090. Full Text via CrossRef | Abstract + References in Scopus | Cited By in Scopus

Ocké et al., 1997 M.C. Ocké, H.B. Bueno-de-Mesquita, H.E. Goddijn, A. Jansen, M.A. Pols, W.A. van Staveren and D. Kromhout, The Dutch EPIC food frequency quentionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groops, Int. J. Epidemiol. 26 (1997) (Suppl. 1), pp. S37–S48. Abstract + References in Scopus | Cited By in Scopus

Patel and Thompson, 2006 M.D. Patel and P.D. Thompson, Phytosterols and vascular disease, Atherosclerosis 186 (2006), pp. 12–19. SummaryPlus | Full Text + Links | PDF (127 K)

Sanders et al., 2000 D.J. Sanders, H.J. Minter, D. Howes and P.A. Hepburn, The safety evaluation of phytosterol esters. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat, Food Chem. Toxicol. 38 (2000), pp. 485–491. Abstract | PDF (228 K) | Abstract + References in Scopus | Cited By in Scopus

SCF, 2002a SCF (Scientific Committee on Food) 2002a. General view on the long-term effects of the intake of elevated levels of phytosterols from mutiple dietary sources, with particular attention to the effects on ß-carotene. Oinion adopted by the Scientific Committee on Food on 26 September 2002.

SCF, 2002b SCF (Scientific Committee on Food) 2002b. Opinion on a report on Post Launch Monitoring of yellow fat spreads with added phytosterol esters. Opinion adopted by the Scientific Committee on Food on 26 September 2002.

Simons, 2002 L.A. Simons, Additive effect of plant sterol-ester margarine and cerivastatin in lowering low-density lipoprotein cholesterol in primary hypercholesterolemia, Am. J. Cardiol. 90 (2002), pp. 737–740. SummaryPlus | Full Text + Links | PDF (125 K) | Abstract + References in Scopus | Cited By in Scopus

Slesinski et al., 1999 R.S. Slesinski, D. Turnbull, V.H. Frankos, A.P. Wolterbeek and D.H. Waalkens-Berendsen, Developmental toxicity study of vegetable oil-derived stanol fatty acid esters, Regul. Toxicol. Pharmacol. 29 (1999), pp. 227–233. Abstract | PDF (60 K) | Abstract + References in Scopus | Cited By in Scopus

Turnbull et al., 1999a D. Turnbull, V.H. Frankos, W.R. Leeman and D. Jonker, Short-term tests of estrogenic potential of plant stanols and plant stanol esters, Regul. Toxicol. Pharmacol. 29 (1999), pp. 211–215. Abstract | PDF (57 K) | Abstract + References in Scopus | Cited By in Scopus

Turnbull et al., 1999b D. Turnbull, V.H. Frankos, J.H. van Delft and N. DeVogel, Genotoxicity evaluation of wood-derived and vegetable oil-derived stanol esters, Regul. Toxicol. Pharmacol. 29 (1999), pp. 205–210. Abstract | PDF (55 K) | Abstract + References in Scopus | Cited By in Scopus

Turnbull et al., 1999c D. Turnbull, M.H. Whittaker, V.H. Frankos and D. Jonker, 13-week oral toxicity study with stanol esters in rats, Regul. Toxicol. Pharmacol. 29 (1999), pp. 216–226. Abstract | PDF (75 K) | Abstract + References in Scopus | Cited By in Scopus

Waalkens-Berendsen et al., 1999 D.H. Waalkens-Berendsen, A.P. Wolterbeek, M.V. Wijnands, M. Richold and P.A. Hepburn, Safety evaluation of phytosterol esters. Part 3. Two-generation reproduction study in rats with phytosterol esters—a novel functional food, Food Chem. Toxicol. 37 (1999), pp. 683–696. SummaryPlus | Full Text + Links | PDF (268 K) | Abstract + References in Scopus | Cited By in Scopus

Weststrate and Meijer, 1998 J.A. Weststrate and G.W. Meijer, Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects, Eur. J. Clin. Nutr. 52 (1998), pp. 334–343. Abstract + References in Scopus | Cited By in Scopus

Weststrate et al., 1999 J.A. Weststrate, R. Ayesh, C. Bauer-Plank and P.N. Drewitt, Safety evaluation of phytosterol esters. Part 4. Faecal concentrations of bile acids and neutral sterols in healthy normolipidaemic volunteers consuming a controlled diet either with or without a phytosterol ester-enriched margarine, Food Chem. Toxicol. 37 (1999), pp. 1063–1071. SummaryPlus | Full Text + Links | PDF (154 K) | Abstract + References in Scopus | Cited By in Scopus

Wolfreys and Hepburn, 2002 A.M. Wolfreys and P.A. Hepburn, Safety evaluation of phytosterol esters. Part 7. Assessment of mutagenic activity of phytosterols, phytosterol esters and the cholesterol derivative, 4-cholesten-3-one, Food Chem. Toxicol. 40 (2002), pp. 461–470. SummaryPlus | Full Text + Links | PDF (124 K) | Abstract + References in Scopus | Cited By in Scopus