Atherosclerosis 214 (2011) 225–230

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Effects of long term plant sterol and -stanol consumption on the retinal vasculature: A randomized controlled trial in statin users

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ARTICLE INFO

Article history:
Received 24 August 2009
Received in revised form 14 October 2010
Accepted 25 October 2010
Available online 2 November 2010

Keywords:
Cholesterol-lowering treatments
Stanol
Sterol
Retina
Arteriole
Venule

ABSTRACT

As sitosterolemic patients have an increased cardiovascular risk, there is concern that reducing serum LDL-cholesterol concentrations by plant sterols enriched functional foods might adversely affect vascular function. Whether increased concentrations of plant sterols truly affect vascular function and whether these effects are exclusive to the larger vessels remains unknown. We compared the effects of long-term plant sterol and -stanol consumption on changes in retinal vessels diameter which reflex alterations in the microcirculation. Three randomized groups were studied at baseline and after 85-weeks. Group one ($N = 11$) consumed plant sterol enriched margarine (2.5 g/day), the second ($N = 8$) plant stanol enriched margarine (2.5 g/day), and the control group ($N = 11$) non-enriched margarine (2.5 g/day). Serum cholesterol-standardized campesterol and sitosterol concentrations increased by $354.84 \pm 168.22 \cdot 102$ μmol/mmol and $84.36 \pm 48.26 \cdot 102$ μmol/mmol ($p < 0.001$), respectively in the sterol group, while decreasing non-significantly in the plant stanol group. Serum LDL-cholesterol concentrations decreased significantly in both the plant sterol (-0.33 ± 0.33 mmol/L, $p = 0.016$) and -stanol groups (-0.38 ± 0.34 mmol/L, $p = 0.018$) compared to the increase in the controls (0.29 ± 0.34 mmol/L). The mean change in venular diameters for the plant sterol group (2.3 ± 3.1 μm), plant stanol groups (-0.8 ± 3.4 μm) and control group (-0.8 ± 5.1 μm) did not reach significance but the change in cholesterol-standardized campesterol concentrations correlated positively with the change in venular diameter independent of changes in serum LDL-cholesterol concentrations ($r = 0.39$, $N = 30$, $p = 0.033$). Increased serum campesterol concentration correlated positively with increased retinal venular diameter, independent from changes in serum LDL-cholesterol concentrations. This may constitute an explanation for the suggested effects of plant sterols on vascular function. However, this novel finding needs confirmation and further study.

1. Introduction

Both plant sterol and -stanol esters are currently used as functional food ingredients to reduce elevated serum LDL cholesterol concentrations. These effects have been shown in numerous population groups – both in short- and long-term placebo controlled intervention studies [1]. As a result, these products have obtained a prominent position within guidelines to lower cardiovascular disease risk [2]. However, some animal and human studies indicate that slightly elevated serum plant sterol concentrations may be atherogenic [3–6]. The basis behind this concern lies in findings of premature coronary heart disease (CHD) in patients with sitosterolemia, which are characterized by severely elevated serum plant sterol concentrations. Recently, several large cross-sectional and prospective cohort studies have therefore tried to link CHD mortality rates to serum plant sterol concentrations and smaller retinal arteriolar diameter. While some studies showed a positive relationship between serum plant sterol concentrations and CHD risk [7–9], others did not [10–12]. Altogether these findings are as yet too inconsistent to draw any definitive conclusions in this respect. Moreover, very recently after many years of debate and careful weighing scientific data the Canadian authorities decided to allow plant sterol enriched products on the Canadian market. This suggests that the expected health improvements might outweigh the suggested atherogenicity. We here propose that this potential atherogenicity might originate from effects on the microcirculation. Although cardiovascular disease is largely considered a disease of the large vessels, evidence is accumulating that disturbances in the microcirculation predict CHD risk [13]. Several studies have indeed shown that a larger retinal venular diameter associates with established markers for atherosclerosis [14–16]. For example, in the Rotterdam Study subjects with larger venular diameters had lower serum HDL cholesterol concentrations, higher serum total cholesterol concentrations, a lower ankle–arm index, a higher carotid-plaque score, and more aortic calcifications [14].

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0021-9150
doi:10.1016/j.atherosclerosis.2010.10.038

In addition, Wong et al. have shown that certain retinal microvascular characteristics predict the incidence of stroke [17]. Finally, Hubbard et al. showed that a decreased arteriovenous ratio (AVR) of 0.02 units predicts a 10 mmHg increase in mean arterial blood pressure (MABP) [18]. The relation between retinal microvascular abnormalities and hypertension was later confirmed by Wong et al., although they found no relation with atherosclerosis in their study [19].

To the best of our knowledge, there have been no studies evaluating or even monitoring the association between serum plant sterol concentrations, nor the effects of plant sterol or -stanol consumption on microvascular characteristics. Plant stanols are the hydrogenated forms of plant sterols. These not only lower serum LDL-cholesterol concentrations, but also that of the serum plant sterols [20–22]. Therefore, in the present study we examined the association between changes in serum plant sterol concentrations and changes in retinal vessel diameter during an 85-week follow-up study in which plant sterol or -stanol esters were consumed. All subjects were already on stable statin treatment for several years and consumed margarines enriched with either plant sterol esters (2.5 g/day), -stanol esters (2.5 g/day), or not enriched at all (control). We have reported earlier that plant sterol or -stanol ester consumption lowered serum LDL cholesterol concentrations in this population over the intervention period of 85 weeks by approximately 10% [23]. We now describe the effects of these interventions on changes in the retinal vasculature diameter in relation to changes in serum LDL cholesterol and cholesterol-standardized plant sterol concentrations. These are secondary outcomes from a larger study.

2. Materials and methods

2.1. Subjects

Subjects were recruited through local newspaper advertisements and posters in the University and hospital buildings. Inclusion criteria were: current treatment with a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor (statin), age 18–65 years, body mass index (BMI) $\leq 32\,kg/m^2$, no proteinuria or glucosuria, diastolic blood pressure ≤ 95 mmHg and systolic blood pressure ≤ 200 mmHg. Since one of the purposes of this study was to analyze the long-term effects of plant sterol or -stanol esters consumption on serum lipoprotein metabolism [24], and on markers of endothelial dysfunction and arterial stiffness in patients being treated stably with statin (to be published elsewhere), statin use was an inclusion criterion. Exclusion criteria were antihypertensive medication use, clinical manifestations of liver disorders, existing diabetes mellitus type 2 or demonstration of cardiovascular or cerebral events within a period of 6 months prior to the study.

2.2. Ethics

The protocol was approved by the Medical Ethical Committee of the Maastricht University, and was conducted in accordance with the Helsinki Declaration. Informed consent was gained from all subjects.

2.3. Diet and design

Subjects were asked to replace their regular margarine or butter with the experimental 'light' margarines (40% fat) supplied by us. They were instructed to consume 30 g/day of the margarine divided over at least two meals during the day. During the run-in period of 5-weeks, all subjects used the control margarine without added plan-sterols or -stanols. At the end of the run-in period, subjects were randomly allocated to one of the three experimental groups, stratified for sex and age in this double blinded intervention trial. For the following 85 weeks (2003–2004), the first group ($N = 11$) continued with the control margarine, while the other two groups used the same margarine, but now enriched with either plant sterols (2.5 g plant sterols/day; $N = 11$) or plant stanols (2.5 g plant stanols/day; $N = 8$). Plant sterols and -stanols were provided as fatty acid esters obtained by transesterification of free plant sterols with sunflower oil based fatty acids (Unilever, Vlaardingen, The Netherlands) or of free plant stanols with rapeseed oil based fatty acids (Walter RauNeusser Öl und Fett AG, Neuss, Germany) [24]. The volunteers came to the university at least once a month to receive their products. A diary was kept by the subjects to record any signs of illness, change in medication, and amount of daily consumed margarine. For the entire study-duration subjects were asked to keep their normal diet, physical exercise level, and smoking and alcohol consumption habits. Details of this study have already been published [23].

2.4. Measurements

2.4.1. Serum lipids, plant sterols and plant stanols

Serum lipids and lipoproteins as well as serum plant sterol and -stanol concentrations at the beginning and the end of the experimental period were analyzed as described [23].

2.5. Fundus photography

Retinal images were taken at baseline (week 5) and at the end of the experimental period (week 90). Mydriasis was achieved using 0.5% tropicamide and 2.5% phenylephrine. The fundus images were taken using an analogue camera and were later digitized. Image-editing software was used to remove the red (containing mostly choroid information) and blue channels (containing no real useful information) from the images leaving only the green light channel. Removal of these two color-channels increases the contrast of the retinal vessels in the remaining image [25,26].

Retinal images were registered using the Generalized Dual-Bootstrap Iterative Closest Point (GDB-ICP) software developed by Stewart and coworkers from the Rensselaer Polytechnic Institute (NY, USA). This registration process has been explained in detail elsewhere [27]. In short, the registration automatically initializes and individually matches vascular landmarks. The images are aligned based on detected vessel key-points using a quadratic transformation with sub-pixel accuracy. The registration enabled us to compare baseline with endpoint images accurately.

The registration was validated using the Retinal Analysis Optimate System developed by Hubbard and colleagues from the University of Wisconsin (WI, USA) and was considered to be valid if the AV-ratio of the original image and the AV-ratio of the registered image did not differ significantly, thus suggesting that no distortion took place during the registration process [14,18]. Note that in the cross-sectional studies published so far, registration was not required since in these types of studies, no comparisons of images within subjects were made for different time points.

Measurements of the retinal vessel diameter were performed using the same Retinal Analysis Optimate System as mentioned before. For the retinal vessels diameter, the mean of the four largest arteries or the four largest veins was taken. The vessels type classification made by the program was confirmed by an independent ophthalmologist.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 17.0 for windows. Differences in gender distribution over the experimental groups were tested using the Pearson Chi-square test, while differences in

age and lipids were evaluated using the ANOVA. The Students' *T*-test was used to evaluate the difference in serum LDL cholesterol concentrations between subjects with ($N = 30$) and without ($N = 17$) retinal images taken. An ANCOVA with follow-up as dependent and baseline levels and changes in sterols and stanols as independent variables was used to evaluate the relation between retinal vessel characteristics and serum sterol concentration. *p*-Values were considered significant if $p < 0.05$. For post hoc test we applied the Bonferroni correction. Results are shown as mean ± standard deviation (SD).

3. Results

3.1. Baseline characteristics

For this study 54 subjects, who met all the inclusion criteria, were recruited. These subjects were randomized into the three groups. Because the number of subjects that we could transport to the testing facilities for fundus photography (located 200 km from our university buildings) was limited, only 46 subjects were able to participate in the first retina photograph session. Of these 46 subjects, only 43 subjects completed the entire study of 85 weeks. In addition, one subject with a change in serum total cholesterol concentrations of almost five SD's from the mean value was considered an outlier and as such excluded from further analysis. Two more subjects failed to attend the second photograph session after 85 weeks, while five subjects were excluded from the statistical analysis because they were requested by their physician to change their dosage of statins during the 85 weeks follow-up period. Finally, retinal images for five subjects were also excluded because of poor quality. Thus, data of 30 subjects could be used, of which eleven (36.7%) participated in the sterol group, eight (26.7%) in the stanol group and the remaining eleven in the control group. Table 1 shows the baseline characteristics. There was no significant difference in gender distribution between the three groups ($p = 0.305$), and no significant differences in age, body-mass-index or blood pressure between the three groups at baseline. There were no adverse effects reported.

3.2. Baseline serum lipids, lipoproteins, plant sterols and plant stanols

Serum concentrations for lipids and lipoproteins and the cholesterol-standardized plant sterol and -stanol concentrations at the end of the run in period (week 4/5) are shown in Table 2. There were no significant differences in the statin type used in the three groups ($p = 0.846$, data not shown). There were no significant differences in serum total cholesterol concentrations between the three groups ($p = 0.335$). Also for HDL cholesterol, triacylglycerol and LDL concentrations there were no significant differences ($p = 0.929$, 0.192 and 0.733, respectively). At baseline, serum-cholesterol-standardized-campesterol ($p = 0.035$) and -sitosterol ($p = 0.049$) concentrations differed significantly between the sterol and stanol group but they did not differ from the control group. No significant differences were observed between serum-cholesterol-standardized-campestanol and sitostanol concentrations at baseline.

A comparison was made between the 30 subjects used in the retinal analysis and those not participating ($N = 17$) to ensure that they had similar cholesterol concentrations at baseline and showed no significant difference for the control, sterol and stanol group ($p = 0.963$, 0.324, and 0.917, respectively). This suggests that the 30 subjects who were finally included in the analysis were a valid representation of the entire group.

Table 2
Baseline concentrations and mean changes for serum lipids and lipoproteins, lathosterol, plant sterols and -stanols.

Group	Baseline	Follow up	Change	% Change
Total cholesterol				
Sterol	5.19 ± 0.88	4.95 ± 0.72	−0.24 ± 0.39	−3.9
Stanol	5.68 ± 0.54	5.36 ± 0.46	−0.32 ± 0.41	−5.4
Control	5.16 ± 0.88	5.45 ± 0.86	0.29 ± 0.34	5.9
p-Value	0.335	0.25	0.002	
LDL cholesterol				
Sterol	3.22 ± 0.68	2.90 ± 0.61	−0.32 ± 0.33	−9.2
Stanol	3.42 ± 0.48	3.09 ± 0.49	−0.34 ± 0.34	−9.6
Control	3.19 ± 0.77	3.33 ± 0.68	0.13 ± 0.35	5.3
p-Value	0.733	0.292	0.006	
HDL cholesterol				
Sterol	1.28 ± 0.34	1.42 ± 0.32	0.14 ± 0.10	12.4
Stanol	1.29 ± 0.18	1.46 ± 0.32	0.17 ± 0.29	13.5
Control	1.32 ± 0.20	1.35 ± 0.21	0.03 ± 0.17	2.9
p-Value	0.929	0.681	0.219	
Lathosterol				
Sterol	56.54 ± 24.11	71.96 ± 33.34	15.42 ± 13.55	27.3
Stanol	58.02 ± 18.54	65.04 ± 21.76	7.02 ± 20.67	12.1
Control	67.72 ± 23.72	60.19 ± 19.60	−7.53 ± 6.30	−11.1
p-Value	0.474	0.574	0.002	
Campesterol				
Sterol	368 ± 181	773 ± 284	405 ± 172	110
Stanol	196 ± 97	168 ± 64	−28 ± 99	−14.3
Control	248 ± 122	238 ± 114	−9.63 ± 32	−3.9
p-Value	0.035	<0.001	<0.001	
Sitosterol				
Sterol	233 ± 111	336 ± 120	103 ± 41	44.2
Stanol	133 ± 65	123 ± 50	−9.03 ± 46	−6.8
Control	170 ± 69	174 ± 77	3.79 ± 33	2.2
p-Value	0.049	<0.0001	<0.0001	
Campestanol				
Sterol	4.06 ± 1.33	9.22 ± 1.95	5.16 ± 1.44	127
Stanol	3.11 ± 1.05	43.8 ± 32	40.7 ± 31	1309
Control	3.29 ± 0.74	7.97 ± 1.66	4.68 ± 1.01	142
p-Value	0.126	<0.0001	<0.0001	
Sitostanol				
Sterol	3.33 ± 1.16	2.78 ± 0.64	−0.55 ± 0.69	−16.5
Stanol	2.74 ± 1.54	20.1 ± 17	17.4 ± 16	635
Control	2.94 ± 0.80	2.93 ± 0.80	−0.01 ± 0.73	−0.3
p-Value	0.536	<0.0001	<0.0001	

Analysis of variance: serum cholesterol concentrations are presented as means ± SD mmol/L. LDL and HDL cholesterol are means ± SD for cholesterol-standardized mmol/L. Plant sterol and -stanol values are cholesterol-standardized (means ± SD × 10^2 μmol/mmol cholesterol).

Table 1
Baseline characteristics of the subjects in the three groups.

	Gender		Age (years)	BMI (kg m^{-2})	Blood pressure (mmHg)		Hypertensive $S > 140$ or $D > 90$ mmHg
	Male	Female			Systolic	Diastolic	
Sterol	6	5	59 ± 9	25.6 ± 2.2	141.5 ± 19.0	87.0 ± 11.1	7
Stanol	3	5	63 ± 6	26.1 ± 2.1	135.9 ± 13.6	82.5 ± 8.0	7
Control	8	3	60 ± 7	25.6 ± 2.5	144.3 ± 18.7	87.4 ± 10.3	4
p-Value	0.305	0.591	0.863	0.594	0.538	0.797	

Values are means ± standard deviation.

3.3. Effect of dietary plant sterols and stanols on serum LDL cholesterol concentrations

The plant sterol group had a 0.33 mmol/L (9.7%; $p = 0.021$) greater decrease in serum LDL cholesterol concentration compared to the control group over the entire 85 week intervention. In the plant stanol group serum LDL cholesterol concentrations were reduced by 0.38 mmol/L (11.2%; $p = 0.008$). Measurements of lathosterol concentrations revealed a significant increase between the sterol group and the control group ($p = 0.002$ in a post hoc analysis).

3.4. Effect on serum cholesterol-standardized plant sterol and -stanol concentrations

In the plant sterol group, cholesterol-standardized campesterol ($p < 0.001$) and sitosterol ($p < 0.001$) concentrations increased significantly as compared to the control and the plant stanol group, while no significant effect on serum campestanol and sitostanol concentrations was seen. The plant stanol group had a significant lower baseline value for campesterol and sitosterol as compared to the plant sterol group ($p < 0.05$). A reduction in the cholesterol-standardized campesterol and sitosterol concentrations in the plant stanol group as compared to the control group did not reach significance. Plant stanol consumption increased the serum cholesterol-standardized campestanol and sitostanol concentrations significantly ($p < 0.001$ in a post hoc analysis) as compared to both the sterol and control groups (see Table 2).

3.5. Retinal arteriolar and venular diameters

Table 3 shows the baseline values as well as the changes in the diameter of the retinal arterioles and retinal venules during the 85-week study duration. Changes in the diameter of both the retinal arterioles and venules were not significantly different between the three dietary interventions ($p = 0.77$ and $p = 0.15$, respectively). However, for the retinal venular diameter, although not statistically significant, there was an increase in mean diameter over time in the plant sterol group as compared to the decrease observed in the other two groups (Table 3).

Since there was a significant difference in serum cholesterol-standardized plant sterol concentrations between the groups at baseline, we also used ANCOVA with the follow-up venular diameters as dependent variable and mean baseline venular diameters and changes in cholesterol-standardized serum-plant sterol or -stanol concentrations as independent variables (Table 4). These results indicate a significant contribution of both the baseline ($\beta = 0.98$, $p < 0.001$) as well as the change in cholesterol-standardized serum campesterol concentrations ($\beta = 0.0066$, $p = 0.049$) on the follow-up venular diameters (model A). In this model we used the mean venular diameter per subject as dependent variable. A more detailed ANCOVA analysis (model B), wherein all four veins of the individual subject were clustered by subject (instead of the mean of the four venules used before) resulted in the same outcome for changes in serum campesterol concentrations ($\beta = 0.0062$, $p = 0.032$). Adding lathosterol to the statistical models did not reveal a significant contribution of changes in lathosterol concentrations to the observed increase in venular diameter. To examine the potential relationship between changes in serum cholesterol-standardized plant sterol concentrations and venular diameter in more detail, we searched for associations between changes in venular diameter and changes in serum lipid, lipoprotein and cholesterol-standardized plant sterol or stanol concentrations (Fig. 1). We found that an increase in serum plant sterol concentrations was associated with a significant increase in venular diameter ($r = 0.387$, $p = 0.034$). Further analysis showed that this significant correlation could be attributed primarily to the change in serum cholesterol-standardized campesterol concentrations ($r = 0.39$, $p = 0.033$) in the sterol group. For

Table 4
Multiple linear regression analysis.

Model	Variables		Regression coefficient		
	Dependent	Independent	*B*	SE (β)	*p*-Value
A[a]	Mean follow-up venular diameter	Mean baseline venular diameter	0.98	0.55	<0.001
		Δ cholesterol-standardized campesterol concentration	0.0066	0.0032	0.049
		Intercept	1.93		
B[b]	Follow-up venular diameter (clustered)	Mean baseline venular diameter	0.98	0.018	<0.001
		Δ chol stand Campesterol conc	0.0062	0.0027	0.032
		Intercept	1.15		

Total cholesterol, LDL cholesterol, and triglycerides did not contribute significantly in the initial model, and therefore they were not included in the final model.
[a] Mean venular-diameter per subject as dependent variable.
[b] All veins included, clustered by subjects (i.e. four per subjects).

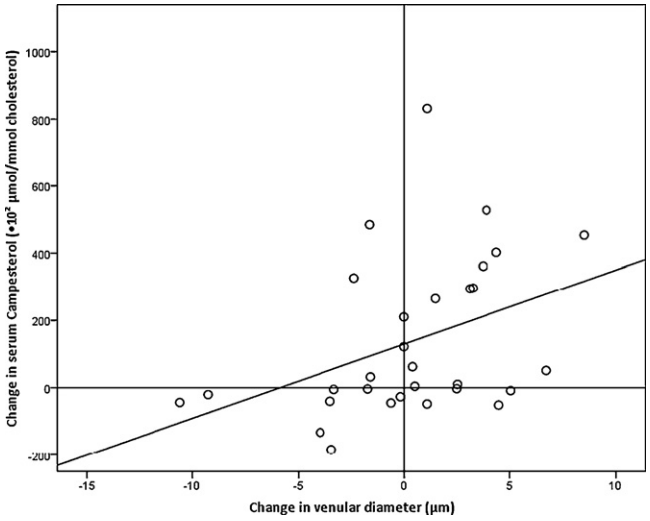

Fig. 1. Scatter plot showing association between change in serum campesterol concentration and change in venular diameter with regression line ($r = 0.39$, $p = 0.033$).

Table 3
Baseline values and mean changes in retinal vessel diameter for the 3 groups.

	Arteriole		Venule		AV-ratio	
	Baseline	Diff	Baseline	Diff	Baseline	Diff
Sterols	91 ± 18.5	−0.7 ± 4.7	106 ± 15	2.3 ± 3.1	0.86 ± 0.13	−0.03 ± 0.05
Stanols	89 ± 8.4	0.6 ± 3.1	109 ± 14	−0.8 ± 3.4	0.83 ± 0.08	0.01 ± 0.03
Controls	89 ± 8.3	−0.2 ± 3.4	111 ± 13	−0.8 ± 5.1	0.81 ± 0.11	0.00 ± 0.04
p-Value	0.931	0.766	0.705	0.149	0.574	0.077

Values are shown as µm means ± standard deviation.

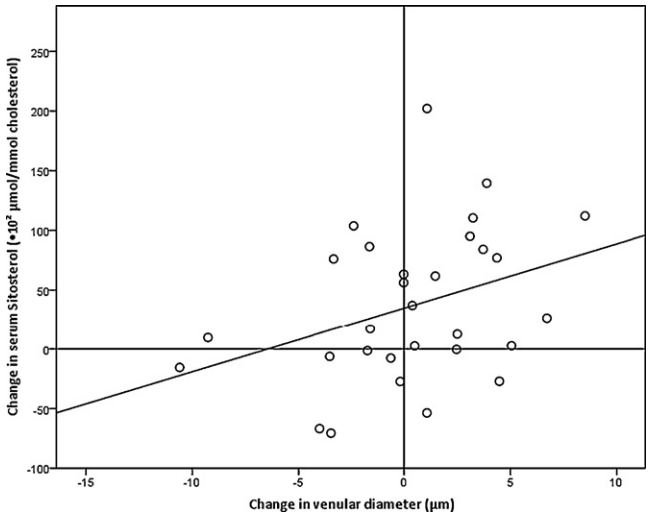

Fig. 2. Scatter plot showing association between change in serum sitosterol concentration and change in venular diameter with regression line ($r = 0.35$, $p = 0.056$).

cholesterol-standardized sitosterol concentrations this correlation nearly reached statistical significance ($r = 0.35$, $p = 0.056$, Fig. 2). There was no correlation between AV-ratio and serum campesterol and sitosterol changes ($p > 0.661$).

As expected, the change in serum cholesterol-standardized plant sterol concentrations (both sitosterol as well as campesterol) showed a significant correlation with the change in serum cholesterol concentrations. However, the changes in retinal vein diameters showed only a relation with changes in cholesterol-standardized serum campesterol concentrations but not with changes in serum cholesterol concentrations. There were also no relations between changes in serum cholesterol-standardized plant stanol concentrations and retinal vein diameters. This indicates that the observed changes in retinal veins are more closely related to the increased plant sterol concentration and not to the decrease in serum-LDL-cholesterol concentrations.

4. Discussion

To the best of our knowledge we present here, for the first time, the effect of increased plant sterol- and stanol-enriched functional food consumption over time on the retinal vasculature. Previous studies have shown an association between retinal vasculature changes and markers for atherosclerosis or future CHD risk [14,28]. A recent study by Kreis et al. failed however to show any significance between retinal vascular caliber and the severity of coronary artery disease [29]. Unfortunately we do not have the statistical strength to stratify for gender as McGeechan et al. showed that especially women with wider retinal vein caliber had an increased hazard ratio for CHD [30]. In our study, the group that consumed plant sterols showed an increase in venular diameter over time, which did not reach statistical significance when compared to the changes in the other two groups. Linear regression analysis indicated however that there was a positive association between an increase in cholesterol-standardized serum campesterol concentrations and an increase in venular diameter. The plant stanol group had a lower baseline sterol level, leading probably to a smaller change in these values. Interestingly, the effects on venular diameter were independent from changes in serum cholesterol, LDL, HDL, or triglyceride concentrations. This may also explain why we found no effects on venular diameter in the plant stanol ester treated group, although serum total and LDL cholesterol concentrations were lowered by a comparable percentage as in the plant sterol ester group. If true, it might be expected for example that sitosterolemic patients – who are characterized by normal serum LDL cholesterol concentrations, severely elevated serum plant sterol concentrations, and a pronounced increase in CHD risk – would be characterized by a severely disturbed microvasculature system [31]. This has however never been evaluated in this patient group.

Although suggestive, it remains to be investigated what the functional consequences of the observed increase in venular diameter in terms of changed cardiovascular risk are. In this respect, Kawasaki et al. showed that subjects with metabolic syndrome had a mean venular diameter, which was 4.69 μm larger than that observed in healthy subjects [32]. Ikram et al. found that for every point increase in carotid plaque-score there was an 0.4 μm increase of the venular diameter, while every SD decrease in ankle–arm index resulted in an increase of 1.3 μm of the venular diameter [14]. They also showed that current smokers had on average a 10 μm larger venular diameter than nonsmokers after adjusting for age and gender. In the light of those findings, the increase in venular diameter of 2.3 μm we observed in the plant sterol group as compared to the decrease in both the control and plant stanol group is at least worth exploring. In contrast to other studies, we did not find any correlation between retinal venular diameter and other established CHD risk factors.

The correlation between the increase in venular diameter and increase in cholesterol-standardized sitosterol concentrations almost reached statistical significance ($p = 0.056$). A possible explanation for the lower significant association between venular diameters and serum cholesterol-standardized sitosterol concentrations as compared to campesterol concentrations might be found in the height of serum concentrations, especially after consumption of the sterol ester enriched products. In the group consuming plant sterol ester enriched foods, the increase in cholesterol-standardized serum campesterol concentrations was four times larger than that of sitosterol ($405 \pm 172 \times 10^2$ μmol/mmol and $102 \pm 41 \times 10^2$ μmol/mmol, respectively; see Table 2). The association between changes in serum cholesterol-standardized sitosterol concentrations and changes in venular diameter did not reach significance. However it might still be possible that the observed changes in mean venular diameter in the plant sterol group we observed can be attributed to an increase in plant sterols in general and not only to campesterol.

It is generally accepted that plant stanol consumption lowers serum plant sterol concentrations by 10–42% [20–22]. This effect is attributed to the ability of plant stanols to lower not only the intestinal absorption of cholesterol but also that of plant sterols. Therefore consumption of plant stanols lowers not only serum cholesterol but also serum plant sterol concentrations. In line with previous studies we found a mean reduction of cholesterol-standardized serum campesterol concentrations of $28 \pm 99 \times 10^2$ μmol/mmol (14.3%) and $9 \pm 47 \times 10^2$ μmol/mmol (6.8%) for sitosterol in the plant stanol esters group. No reduction in venular diameter was observed. However, it is possible that the reduction in serum cholesterol-standardized plant sterol concentrations induced by plant stanol consumption is too small to be protective or have any effect at all. This however demands further study.

An intriguing final question that remains is how to explain the observed effects of elevated cholesterol-standardized campesterol concentrations – and maybe also those of sitosterol – on the retinal venular diameter. One possible explanation might relate to currently unknown regulatory effects of plant sterols or one of its metabolites within the wall of the retinal venules, which is not seen with plant stanols or cholesterol. We do know for example that plant sterols may oxidize into various oxidation products [33]. The reason that plant stanols do not show this effect might relate to the absence of formation of the responsible metabolites from plant stanols, or otherwise to the lower serum concentrations of plant stanols as compared to plant sterols. Nagaoka et al. showed

that statin treatment affects retinal blood flow in both arterioles and venules [34]. In a later study they reported that effects of simvastatin on retinal arterioles were dose-dependent and nitric oxide-mediated [35]. Although of interest, effects on the retinal venular diameter we observed in our study are independent of statin use since all groups were on statin treatment for already two years prior to being recruited in this trial and remained during the entire study period.

To summarize, we have shown that an increase in cholesterol-standardized serum campesterol concentrations observed during long-term consumption of plant sterol ester enriched functional foods correlates with increase in retinal venular diameter. The functional consequences of the increase in retinal venular diameter in terms of affecting health demands further study, although the observed increase of 2.3 μm is certainly relevant when placed in perspective to associations found in for example metabolic syndrome subjects and smokers.

We concluded that the positive correlation between the increases in serum campesterol concentrations and increases in retinal venular diameter – which was independent from changes in serum LDL cholesterol concentrations – may be an explanation for the suggested adverse effects of plant sterols on vascular function. This is however a biomarker and more evidence is needed that plant sterol consumption indeed affects the functioning of our (micro)vascular system. We are aware that this is the first time this relation has been studied and therefore it certainly needs confirmation. Therefore, clearly further studies are warranted before complete safety of functional foods containing plant sterols can be judged.

Competing interests

The authors have no proprietary interest in any aspect of this report.

Source of Funding

This project was funded by the Netherlands Organization for Health Research and Development (Program Nutrition: Health, Safety and Sustainability, grant 014-12-010). The products were delivered free of charge by the companies mentioned under the method section. The authors declare to have received no compensation for their work in any way by the product suppliers. J. Plat and R.P. Mensink planned the study. T.T.J.M. Berendschot collected the data. T.T.J.M. Berendschot and E.R. Kelly analyzed the data and drafted the article. J. Plat, R.P. Mensink reviewed and commented on the manuscript. All authors approved the final version of the manuscript.

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