Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 1241 – 1247
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Preferential campesterol incorporation into various tissues in apolipoprotein E*3-Leiden mice consuming plant sterols or stanols

Jogchum Plat[a,*], Arienne de Jong[a], Oscar L. Volger[b], Hans M.G. Princen[b], Ronald P. Mensink[a]

[a]*Department of Human Biology, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands*
[b]*TNO Gaubius, PO Box 2215, 2301 CE Leiden, The Netherlands*

Received 20 November 2007; accepted 9 April 2008

Abstract

Intestinal absorption of plant sterols and stanols is much lower as compared with that of cholesterol; and therefore, serum concentrations are low. Circulating plant sterols and stanols are incorporated into tissues. However, hardly any data are available about tissue distributions of individual plant sterols and stanols, particularly in relation to their serum concentrations. We therefore fed female apolipoprotein E*3-Leiden mice a control diet, a plant sterol–enriched diet (1g/100 g diet), or a plant stanol–enriched diet (1g/100 g diet) for 8 weeks. In the sterol group, serum cholesterol-standardized campesterol and sitosterol concentrations were, respectively, 8 and 7 times higher as compared with those in the control group. Consequently, the serum campesterol-sitosterol ratio remained essentially unchanged. Cholesterol-standardized plant sterol concentrations increased significantly in all analyzed tissues, except brain. However, the campesterol-sitosterol ratio also increased in all tissues (except in liver and spleen), suggesting that campesterol is preferentially incorporated over sitosterol in those tissues. For the stanol group, serum plant stanol concentrations also increased; but the increase was but less pronounced. We conclude that, in apolipoprotein E*3-Leiden mice, campesterol is preferentially incorporated into most tissues over sitosterol, which cannot be deduced from changes in serum concentrations.

1. Introduction

Plant sterols and stanols are cholesterol-like compounds that reduce intestinal cholesterol absorption and, as a result, lower serum low-density lipoprotein (LDL) cholesterol concentrations. High-density lipoprotein cholesterol concentrations are unaffected. At a daily intake of 2 to 2.5 g, these components lower LDL cholesterol concentrations on average by 9% to 10% [1]. This can be regarded as a favorable effect because an elevated serum LDL cholesterol concentration is an independent risk factor for coronary heart disease. Estimates for the intestinal absorption ranges from 0.4% to 3.5% for plant sterols and from 0.02% to 0.3% for plant stanols [2,3]. In contrast, cholesterol absorption is much higher and varies between 40% and 60% [3,4]. Consequently, plant sterol and stanol concentrations in serum are rather low. Because plant sterols and stanols are transported by lipoproteins, concentrations are normally expressed relative to those of cholesterol. Thus, an increase in the plant sterol (or plant stanol) to cholesterol ratio indicates a relatively higher concentration of plant sterols and a changed composition of the lipoprotein particles. Examples of factors that change the plant sterol to cholesterol ratio are single nucleotide polymorphisms in the ABCG5/G8 transporters [5,6] as well as the use of statins (hydroxymethylglutaryl–coenzyme A reductase inhibitors) [7]. Recently, we have summarized these factors based on a systematic review of all published placebo-controlled human intervention trials [8]. The impact of increased plasma plant sterol concentrations on human health, either positive or negative, is unknown. It is known, however, that part of these circulating plant sterols and stanols is incorporated into tissues. However, hardly any data are available about changes in tissue distributions of individual plant sterols and stanols in relation to changes in their serum concentrations. In addition to the scarce amount of data concerning plant sterols, even less data are available on tissue concentrations of plant stanols in relation to their serum concentrations. Of course, such data are difficult to

* Corresponding author. Tel.: +31 43 3881309; fax: +31 43 3670976.
E-mail address: j.plat@hb.unimaas.nl (J. Plat).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.04.018

obtain from human studies, which makes animal studies necessary. Therefore, the aim of this study was to determine to what extent the various plant sterols and stanols are incorporated into different tissues. We were particularly interested to see whether changes in tissue concentrations of the individual plant sterols (sitosterol/campesterol) or stanols (sitostanol/campestanol) followed those in serum. This latter question may be relevant because, in humans, serum concentrations can easily be measured. To address this question, apolipoprotein (apo) E*3-Leiden transgenic mice were fed a plant sterol– or stanol–enriched diet for 8 weeks, after which plant sterol and stanol patterns were determined in a number of tissues and compared with those in serum.

We here show that campesterol is preferentially incorporated into most tissues over sitosterol, which cannot be deduced from changes in serum concentrations. This also implies that, when considering cellular effects of plant sterols, effects of campesterol should preferably be monitored.

2. Methods

2.1. Design, mice, and diets

We decided to use female apoE*3-Leiden mice for this study because we would like to evaluate sterol and stanol deposition in a situation comparable with the human lipoprotein situation, that is, serum LDL cholesterol higher than high-density lipoprotein cholesterol. This aspect seems important because plant sterols are transported through the circulation as part of the lipoproteins and, in theory, the uptake into the different tissues may depend on the way the sterols are presented to the tissues. In the human situation, serum cholesterol is located in the total apo B fraction for 70% to 80% and in the apo A-I fraction for 20% to 30%, which is also seen in the apoE*3-Leiden model. During a run-in period of 4 weeks, seventeen 3-month–old female apoE*3-Leiden mice were fed a Western-type diet (Table 1) with no additionally added plant sterols or stanols. This control diet contained only plant sterols provided by soy-based oil that is normally used for chow preparation. For the next 8 weeks, the mice were randomly divided into 3 groups. One group of 5 mice (control group) continued with the Western-type diet containing 250 mg cholesterol per 100 g and 37 energy percentage (en%) of fat, of which 15.6 en% was provided as saturated fat, 15.1 en% as monounsaturated fats, and 6.5 en% as polyunsaturated fats; a second group of 6 mice received the same diet enriched with 1 g/100 g plant stanols; and a third group of 6 mice received the same diet enriched with 1 g/100 g plant sterols (Table 1). This high-fat diet was chosen because plant sterol and stanol absorption may depend on the dietary composition because the presence of fat and the consequent bile secretion are essential factors in intestinal plant sterol and stanol absorption. Plant sterols and stanols were provided as their fatty acid esters, obtained by esterification with fatty acids from low erucic rapeseed oil (Raisio Life Sciences, Raisio, Finland). A follow-up period of 8 weeks was chosen because, in general, 4 weeks is needed to reach new stable lipid concentrations, whereas 4 additional weeks was used for reaching new steady-state levels for serum plant sterol and stanol concentrations. The plant stanols were prepared by saturation of a part of the batch of plant sterols that was used for the production of plant sterol esters. The animals were housed in wire-topped cages and fed ad libitum.

Table 1
Composition of the diets

	Diet		
	Control group[a]	Plant stanol group	Plant sterol group
Diet components	g/100 g diet		
Western fat[b]	11	11	11
Sucrose	38.5	38.5	38.5
Corn starch	10	10	10
Casein	20	20	20
Cellulose	5.95	5.95	5.95
Cholesterol	0.25	0.25	0.25
Free sterols/stanols	0.0	1.0	1.0
% of free sterols/stanols			
β-Sitosterol	0.0	1.1	45.5
Campesterol	0.0	0.85	27.0
Stigmasterol	0.0	0.0	17.0
Brassicasterol	0.0	0.0	4.4
Sitostanol	0.0	73.0	2.01
Campestanol	0.0	24.7	0.61

[a] The control diet contained very low amounts of plant sterols (ie, 3.6, 9.5, and 3.5 mg/100 g diet of campesterol, sitosterol, and stigmasterol) derived from 5% soybean oil used in the chow preparation.

[b] Western fat consisted of 40.7% (wt/wt) monounsaturated fat, 17.4% (wt/wt) polyunsaturated fat, and 41.9% (wt/wt) saturated fat. Total fat provides 37.2 en%; sucrose, 35.3 en%; cornstarch, 9.4 en%; and casein, 18.8 en%.

During the study, body weight and food disappearance—expressed as gram per mouse per day—were determined periodically. Blood was collected from the tail vein after 4-hour fasting at week 4 (run-in period) and week 12 (experimental period). Serum was obtained by centrifugation of the blood at 2000*g* and used for plant sterol, stanol, and lipid analyses. At the end of the experimental period, the animals were killed. In this study, we tried to focus on steroid-rich tissues. Examples of this are sterol-metabolizing tissues like adrenals, ovaria, brain, liver, etc, and tissue part of the gastrointestinal tract. Therefore, colon, small intestine, heart, brain, liver, spleen, kidneys, adrenals, and ovaries were removed; extensively rinsed with phosphate-buffered saline at 4°C to remove remainders of feces or blood; snap-frozen in liquid nitrogen; and stored at −80°C until plant sterol and stanol analyses.

2.2. Serum and tissue concentrations or sterols and stanols

Serum total cholesterol and triglyceride concentrations were measured enzymatically by using commercially available kits (236691; Boehringer, Mannheim, Germany, and 337-B, GPO-Trinder kit; Sigma Chemical, St Louis, MO) as

Table 2
Absolute (A) and cholesterol-standardized (B) serum plant stanol and sterol concentrations after 8 weeks of plant stanol ester or sterol ester consumption as compared with control

	Control diet	Plant stanol diet	Plant sterol diet
A. Absolute concentrations			
Cholesterol	11.8 ± 1.6	6.5 ± 1.0 *	6.3 ± 05 *
Sitosterol	14.0 ± 2.0	5.9 ± 0.6 *	51.6 ± 4.8 *,†
Campesterol	79.3 ± 8.5	34.3 ± 1.5 *	355.4 ± 16.7 *,†
Campestanol	0.3 ± 0.0	1.4 ± 0.1 *	0.3 ± 0.0 †
Sitostanol	ND	1.3 ± 0.2	ND
B. Plant sterol-cholesterol ratio			
Sitosterol	1.18 ± 0.17	0.91 ± 0.09	8.13 ± 0.76 *,†
Campesterol	6.70 ± 0.72	5.28 ± 0.23	56.26 ± 2.58 *,†
Campestanol	0.08 ± 0.05	0.20 ± 0.03 *	0.11 ± 0.03 †
Sitostanol	ND	0.21 ± 0.02	ND
Campesterol/Sitosterol ratio	5.71 ± 0.70	5.85 ± 0.45	6.91 ± 0.33

Values are means ± SD and expressed in millimoles per liter for cholesterol or in micromoles for plant sterols (A: absolute concentrations) or $10^2 \times \mu$mol/mmol cholesterol for cholesterol-standardized plant sterols (B: ratios). ND indicates not detectable.

* $P < .05$ vs the control group.

† $P < .05$ vs the stanol group.

described before [9]. Serum sterols and stanols were extracted as described [10], with the following minor modifications. During saponification, the internal standards were evaporated to dryness at 37°C at a moderate nitrogen flow before serum was added. Furthermore, for extraction of sterols and stanols from tissues, some additional steps were required. First, the tissues were homogenized in distilled water and then extracted twice with a chloroform-methanol mixture (2:1 vol/vol). Saponification and calculations related to internal standards were similar as described for serum samples. After extraction, serum and tissue sterols were separated on a 25-m × 0.32-mm capillary GLC column (Altech AT1701; Chrompack, Middelburg, the Netherlands) in an 8000 Top gas chromatograph (CE Instruments, Milan, Italy) equipped with a flame ionization detector (model 980). Quantification was based on the ratio of the area of a particular sterol/stanol to that of 5β-cholest-3-α-ol (C-2882; Sigma Aldrich, St Louis, MO) for the noncholesterol sterols and to that of 5α-cholestane (C-8003) for cholesterol. For protein standardization of tissue plant sterol and stanol levels, the method of Lowry was used. In brief, organs were destructed in sodium hydroxide. After complete destruction, the solution was neutralized with hydrogen chloride and subsequently diluted with distilled water and mixed with a solution containing 2% Na_2CO_3 in 0.1 mol/L NaOH and 0.5% $CuSO_4 \cdot 5H_2O$ in sodium citrate. After 10 minutes of incubation at room temperature, Folin-Ciocalteu phenol reagent (diluted 2 times with distilled water) (Merck, Darmstadt, Germany) was added. Finally, after 30 minutes of incubation at room temperature, the protein concentration was measured spectrophotometrically at 750 nm.

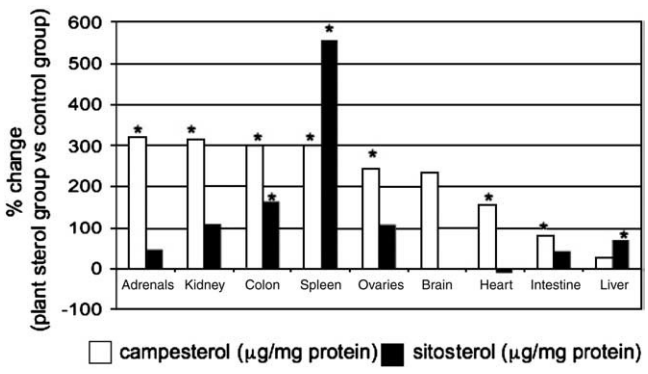

Fig. 1. Changes in tissue campesterol and sitosterol concentrations (in micrograms per milligram protein) in female apoE*3-Leiden mice of the plant sterol ester–fed group as compared with the control group. Changes are expressed as percentage changes vs the control group, and significant differences (*$P < .05$) are marked.

2.3. Statistical analyses

Data were not normally distributed, as evaluated by the Shapiro-Wilk test. Therefore, the nonparametric Kruskall-Wallis test was used to test for statistically significant differences between the 3 groups of mice. When this test indicated a statistically significant difference ($P < .05$), the groups were compared pairwise using the Mann-Whitney test. Data are expressed as means ± SD. All tests were performed with the computer program SPSS 11 for Macintosh (SPSS, Chicago, IL).

3. Results

Plasma total cholesterol concentrations were almost identical for both the plant sterol– and plant stanol–fed groups at the end of the 8-week intervention period, that is, 6.3 ± 0.5 and 6.5 ± 1.0 mmol/L, whereas in the control group, the cholesterol concentration at the end of the intervention period was 11.8 ± 1.6 mmol/L. Body weight of the mice did

Table 3
Absolute sitosterol and campesterol concentrations in various tissues after 8 weeks of plant stanol ester or sterol ester consumption as compared with control

	Control diet	Plant stanol diet	Plant sterol diet
Adrenals			
Sitosterol	0.65 ± 0.14	0.57 ± 0.12	0.95 ± 0.27
Campesterol	3.14 ± 0.25	2.59 ± 0.30 *	13.24 ± 2.80 *,†
Kidney			
Sitosterol	0.04 ± 0.03	0.06 ± 0.03	0.08 ± 0.04
Campesterol	0.13 ± 0.02	0.09 ± 0.02 *	0.56 ± 0.07 *,†
Colon			
Sitosterol	0.06 ± 0.03	0.05 ± 0.03	0.15 ± 0.07 *,†
Campesterol	0.12 ± 0.02	0.10 ± 0.04	0.46 ± 0.12 *,†
Spleen			
Sitosterol	0.01 ± 0.01	0.02 ± 0.02	0.07 ± 0.03 *,†
Campesterol	0.16 ± 0.03	0.11 ± 0.02 *	0.62 ± 0.11 *,†
Ovaries			
Sitosterol	0.15 ± 0.05	0.24 ± 0.01	0.32 ± 0.13
Campesterol	0.42 ± 0.14	0.43 ± 0.14	1.44 ± 0.16
Brain			
Sitosterol	0.10 ± 0.05	0.10 ± 0.05	0.10 ± 0.05
Campesterol	0.16 ± 0.02	0.22 ± 0.08	0.54 ± 0.47
Heart			
Sitosterol	0.05 ± 0.05	0.04 ± 0.03	0.04 ± 0.02
Campesterol	0.14 ± 0.08	0.09 ± 0.03	0.35 ± 0.07 *,†
Small intestine			
Sitosterol	0.04 ± 0.02	0.07 ± 0.04	0.06 ± 0.02
Campesterol	0.18 ± 0.07	0.10 ± 0.05 *	0.32 ± 0.07 *,†
Liver			
Sitosterol	0.04 ± 0.01	0.01 ± 0.00 *	0.06 ± 0.01 *,†
Campesterol	0.55 ± 0.17	0.17 ± 0.04 *	0.71 ± 0.05 †

Values are means ± SD and expressed in micrograms per milligram protein.
* $P < .05$ vs the control group.
† $P < .05$ vs the stanol group.

not significantly change during the study (data not shown). Differences in absolute and cholesterol-standardized serum plant sterol and stanol concentrations are shown in Table 2. Although the intakes of plant sterol or stanols were comparable, cholesterol-standardized serum sitosterol and campesterol concentrations in the plant sterol–fed group were substantially higher than those of sitostanol and campestanol in the plant stanol group. There was an 8-fold increase for cholesterol-standardized serum campesterol concentrations and a 7-fold increase for sitosterol, implying that the serum campesterol-sitosterol ratio did not change substantially during the intervention period. In contrast, in the plant stanol–fed mice, serum cholesterol-standardized campesterol and sitosterol concentrations were lowered by 23% and 21%, respectively, again implying that the sitosterol-campesterol ratio in serum was rather stable. Feeding plant stanol esters increased cholesterol-standardized serum campestanol concentrations by 150%. Sitostanol also increased, but was not detectable in serum of the control as well as the plant sterol group.

Although the cholesterol-standardized levels of sitosterol and campesterol in serum of plant sterol–fed mice increased to the same extent, we observed that the cholesterol-standardized tissue campesterol concentrations were more prominently increased than those of sitosterol (Fig. 1 and Table 3). For campesterol, the concentrations were significantly ($P < .017$) increased—as compared with the control group—in all organs except for the liver and the brain. As shown in Fig. 1, in descending order, the relatively highest campesterol accumulation was found in the adrenals, kidneys, colon, spleen, ovaries, and brain. Cholesterol-standardized sitosterol concentrations were significantly ($P < .017$) increased in the colon, liver, and spleen as compared with the control group. Consequently, the campesterol-sitosterol ratio was increased in all tissues except for the liver and the spleen.

In the plant stanol–fed group, campesterol was significantly decreased in the spleen, liver, and kidney as compared with the control group (Fig. 2). Sitosterol was only significantly ($P = .006$) decreased in the liver. Sitostanol was not detectable in the various tissues, whereas cholesterol-standardized campestanol concentrations were increased in most organs without reaching statistical significance (data not shown). Furthermore, the percentage increases in cholesterol-standardized campestanol concentrations in the plant stanol–fed group were considerably lower (13%-113%) than those for sitosterol (0%-555%) or campesterol (28%-590%) in the plant sterol–fed group.

In summary, the serum campesterol-sitosterol ratio is not changed during the plant sterol or stanol intervention periods, whereas the tissue campesterol-sitosterol ratio is increased during plant sterol intervention in almost all tissues analyzed, indicating preferential incorporation of campesterol.

4. Discussion

As expected, we found that dietary plant sterol and stanol esters lowered serum cholesterol concentrations in apoE*3-Leiden mice. Despite these encouraging results, there is some concern with respect to possible adverse effects of increases in serum plant sterol or stanol concentrations [11-14]. Especially

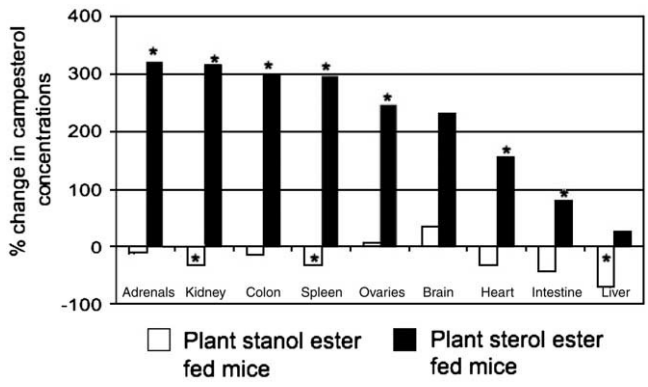

Fig. 2. Changes in tissue campesterol concentrations (in micrograms per milligram protein) of female apoE*3-Leiden mice after 8-week consumption of the plant sterol ester– or plant stanol ester–enriched diets as compared with the control group. Changes are expressed as percentage changes vs the control group, and significant differences (*$P < .05$) are marked.

plant sterols and stanols incorporated in tissues may in theory cause adverse effects that we are currently not aware of. Therefore, it is of utmost importance to know exactly where these compounds are deposited to be able to monitor potential unknown or unexpected effects in the human situation especially when enriched products are consumed.

In the present study, we have now shown that relative changes in serum sitosterol (7-fold) and campesterol (8-fold) concentrations were comparable when apoE*3-Leiden mice consumed a diet enriched with 1% (wt/wt) of a plant sterol mixture, which consisted of 46% sitosterol and 27% campesterol. Like in humans [3], this suggests that campesterol is preferentially absorbed over sitosterol, as serum campesterol concentrations were higher than those of sitosterol, whereas campesterol concentrations in the diets were lower. Although the campesterol-sitosterol ratio in plasma remained essentially unchanged, increases in cholesterol-standardized plant sterol concentrations in various tissues were more pronounced for campesterol, that is, the campesterol-sitosterol ratio increased. This was evident in almost all tissues, except for the liver and the spleen in which predominantly sitosterol was incorporated. Whether these enrichments with plant sterols resulted in any functional changes was not studied. However, it implies that, when discussing cellular effects of plant sterols, it is more likely to study effects of campesterol instead of sitosterol. Safety studies in rats however did not reveal any adverse effects of plant sterol mixtures, even when intakes were well above recommended intakes (reviewed in Katan et al [1]).

In line with findings in human studies [1], cholesterol-standardized serum plant stanol concentrations were much lower than those of plant sterols. In addition, as for the plant sterols, plant stanols were also incorporated into various tissues, but to a much lower extent than plant sterols. Finally, plant stanol consumption decreased plant sterol concentrations in plasma and most tissues in the plant stanol–fed group. This seemed especially true for campesterol. Thus, it seems that especially tissue campesterol concentrations are more readily modifiable by dietary means than those of sitosterol. Interestingly, these changes did not follow those in serum because the campesterol-sitosterol ratio in serum remained essentially the same.

Until now, only a limited number of studies have evaluated the tissue distribution of plant sterols and stanols in (nontransgenic) animals and humans. Bhattacharyya and Lopez [15] studied absorption and accumulation of plant sterols in New Zealand white rabbits after a diet containing 2% of a plant sterol mixture (59.% β-sitosterol, 35% campesterol, and 6% stigmasterol). Interestingly, in the peripheral tissues, such as subcutaneous adipose tissue, muscle, skin, and tendons, only campesterol was present as well as in the aorta. In the abdominal organs, like the liver, small intestine, kidney, adrenal, and ovary, both campesterol and sitosterol were present; but concentrations of campesterol were still higher than those of sitosterol. In rats, Sanders et al [16] showed increases in tissue concentrations of β-sitosterol, sitostanol, campesterol, campestanol, and stigmasterol after a diet (30-40 mg/kg body weight) enriched with a plant sterol/stanol mixture (60% β-sitosterol, 8% campesterol, 8% stigmasterol, and 15% sitostanol). Absorption of campesterol was the highest (13%), followed by β-sitosterol and stigmasterol (4% of the dose) and finally β-sitostanol. Furthermore, plant sterols accumulated in the ovary and adrenal cortex. This elevation was approximately 10-fold higher than that observed in other tissues. In general, campesterol was preferentially incorporated over sitosterol. Unfortunately, plasma plant sterol and stanol concentrations were not reported in this study. In a third study by Kritchevski et al [17], serum and aortic plant sterol concentrations in New Zealand white rabbits were determined after consumption of a plant sterol– or plant stanol–enriched cholesterol-free diet providing 0.27 to 0.30 g of sterol or stanol daily. In the plant sterol–fed group, campesterol was dominantly present, both in serum as well as in aortas. In the plant stanol–fed group, sitostanol increased, whereas both sitosterol and campesterol decreased in aortic tissue. Campestanol was not detected. Finally, Connor and coworkers [18] showed that male Wistar rats consuming plant stanol ester–rich diets (0.5 g stanols per 100 g) resulted in a pronounced incorporation of both sitostanol and campestanol into skeletal muscle, heart muscle, and liver. The finding of sitostanol tissue incorporation is in contrast to our findings in the apoE*3-Leiden mice in which we could not find any sitostanol in the various tissues analyzed. However, this difference might be explained by the fact that serum plant stanol concentrations (especially after consumption of the stanol ester–rich diets) in the rats were several fold higher than those in our mice. Altogether, these animal studies showed preferential campesterol incorporation in subcutaneous fat, muscle, skin, liver, small intestine, kidney, ovary, adrenal cortex, and aorta in 2 different animal models, that is, rats and rabbits. Unfortunately, these earlier studies did not evaluate whether changes in tissue plant sterol or stanol levels were reflected in serum. However, these earlier results are in good agreement with our findings in the apoE*3-Leiden mice. In addition, we also found incorporation of campesterol in the brain. This finding is in line with recent data from mice deficient in ABCG5 or ABCG8, which also showed elevations of plant sterols (both campesterol and sitosterol) in brain. Interestingly, this increase was not observed in apo E knockout mice, suggesting that apo E is needed as a chaperone protein facilitating sterol transport over the blood-brain barrier [19]. This may also explain the incorporation of campesterol in the brains in our apoE*3-Leiden mice. In sitosterolemic patients, Salen et al [20] did not find any plant sterols in the brain; and it was concluded that, in sitosterolemia, the blood-brain barrier is still intact and plant sterols can hardly cross this barrier. In contrast, it has also been reported that [21] sitosterol and campesterol concentrations were slightly increased in the frontal cortex and the basal ganglia of Alzheimer patients as compared with those in healthy controls. Although this latter study shows that these compounds can cross the

blood-brain barrier, it does not demonstrate that plant sterol consumption is causally related to the development of Alzheimer disease.

In contrast to data from animal studies, tissue distribution patterns of plant sterols and stanols in humans are less well defined. Only 3 very small scale (case) studies and a larger study in patients undergoing endarterectomy have described tissue plant sterol concentrations in a selective number of tissues in humans. Bhattacharyya and Connor [22] for the first time found increased plant sterol concentrations in plasma, skin, and tendons (xanthomas) of 2 sisters with sitosterolemia. In both plasma and tissues, sitosterol concentrations were higher than those of campesterol. Mellies et al [23] described plant sterol dispersal in aortic tissues and showed that moderate amounts of plant sterols (mainly campesterol and stigmasterol) were present in atheromatous lesions. This illustrates that, in the absence of sitosterolemia, sitosterol is not the predominant tissue plant sterol. Unfortunately, plasma data for these subjects were not presented. Salen et al [20] compared the sterol tissue patterns of a sitosterolemic patient with that of a healthy control. In this study, in line with the observation of Bhattacharyya and Connor [22] in the sitosterolemic sisters, sitosterol was again dominantly present over campesterol in plasma, erythrocytes, lung, liver, heart muscle, thoracic aorta, and xanthomas of the sitosterolemic patient. It is unclear why, in sitosterolemic patients, more sitosterol is incorporated in tissues. It is possible that it is simply related to the higher plasma concentrations of sitosterol available for the tissues to take up. As already discussed, tissue concentrations in subjects without sitosterolemia do not show this preference for sitosterol incorporation [23]. This may also imply that, in healthy subjects in whom serum campesterol concentrations are higher, predominantly campesterol is present in tissues. In this respect, Miettinen et al [24] have indeed shown that the serum campesterol to cholesterol ratios of patients undergoing endarterectomy correlated positively with the vascular campesterol to cholesterol ratios. Although sitosterol was also detected in atherosclerotic plaques, no results concerning a potential correlation for the sitosterol to cholesterol ratio were presented. This study illustrates that, for some tissues, serum concentrations of campesterol may predict tissue concentrations. Unfortunately, we did not measure plant sterol concentrations in the aortic wall of our mice. Finally, an additional remark is that, from this study of Miettinen et al [24], it cannot be concluded whether the elevated campesterol to cholesterol ratio contributed to atherosclerotic plaque development.

In conclusion, we have shown in apoE*3-Leiden mice that campesterol is preferentially incorporated into most tissues over sitosterol, which cannot be deduced from changes in serum concentrations. In this respect, tissues with the highest incorporation rates were adrenals, kidney, colon, and spleen. It also implies that, when considering cellular effects of plant sterols, preferably effects of campesterol should be monitored. Finally, elevated serum cholesterol-standardized plant stanol concentrations only lead to nonsignificantly elevated cholesterol-standardized campestanol tissue concentrations; but percentage changes in campestanol were less pronounced than those observed for campesterol.

Acknowledgment

Supported by a grant from the Netherlands Organization for Health Research and Development (Program Nutrition: Health, Safety and Sustainability, grant 014-12-010).

We would like to thank Raisio Life Sciences (Finland) for providing the plant sterol and stanol esters used in the mice diets. We acknowledge Frank Cox (Maastricht University, Maastricht) for the plant sterol and plant stanol analyses.

RPM, HMGP, and JP designed and supervised the study. OLV performed the intervention study. AJ statistically analyzed the data. All authors contributed to the writing of the paper. None of the authors had a conflict of interest.

References

[1] Katan MB, Grundy SM, Jones P, Law M, Miettinen T, Paoletti R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 2003;78:965-78.

[2] Heinemann T, Axtmann G, von Bergmann K. Comparison of intestinal absorption of cholesterol with different plant sterols in man. Eur J Clin Invest 1993;23:827-31.

[3] Ostlund Jr RE, McGill JB, Zeng CM, Covey DF, Stearns J, Stenson WF, et al. Gastrointestinal absorption and plasma kinetics of soy Delta (5)-phytosterols and phytostanols in humans. Am J Physiol Endocrinol Metab 2002;282:E911-6.

[4] Wilson MD, Rudel LL. Review of cholesterol absorption with emphasis on dietary and biliary cholesterol. J Lipid Res 1994;35: 943-55.

[5] Berge KE, Tian H, Graf GA, Yu L, Grishin NV, Schultz J, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 2000;290:1771-5.

[6] Plat J, Nichols JA, Mensink RP. Plant sterols and stanols: effects on mixed micellar composition and LXR (target gene) activation. J Lipid Res 2005;46:2468-76.

[7] Miettinen TA, Gylling H. Regulation of cholesterol metabolism by dietary plant sterols. Curr Opin Lipidol 1999;10:9-14.

[8] Chan YM, Varady KA, Lin Y, Trautwein E, Mensink RP, Plat J, et al. Plasma concentrations of plant sterols: physiology and relationship with coronary heart disease. Nutr Rev 2006;64:385-402.

[9] Volger OL, van der Boom H, de Wit EC, van Duyvenvoorde W, Hornstra G, Plat J, et al. Dietary plant stanol esters reduce VLDL cholesterol secretion and bile saturation in apolipoprotein E*3-Leiden transgenic mice. Arterioscler Thromb Vasc Biol 2001;21:1046-52.

[10] Plat J, Mensink RP. Effects of diets enriched with two different plant sterol ester mixtures on plasma ubiquinol-10 and fat-soluble antioxidant concentrations. Metabolism 2001;50:520-9.

[11] Ratnayake WM, Plouffe L, Hollywood R, L'Abbe MR, Hidiroglou N, Sarwar G, et al. Influence of sources of dietary oils on the life span of stroke-prone spontaneously hypertensive rats. Lipids 2000;35: 409-20.

[12] Assmann G, Cullen P, Erbey J, Ramey DR, Kannenberg F, Schulte H. Plasma sitosterol elevations are associated with an increased incidence of coronary events in men: results of a nested case-control analysis of the Prospective Cardiovascular Munster (PROCAM) study. Nutr Metab Cardiovasc Dis 2006;16:13-21.

[13] Miettinen TA, Gylling H, Strandberg T, Sarna S. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of

Scandinavian simvastatin survival study. Finnish 4S Investigators. BMJ 1998;316:1127-30.
[14] Sutherland WH, Scott RS, Lintott CJ, Robertson MC, Stapely SA, Cox C. Plasma non-cholesterol sterols in patients with non–insulin dependent diabetes mellitus. Horm Metab Res 1992;24:172-5.
[15] Bhattacharyya AK, Lopez LA. Absorbability of plant sterols and their distribution in rabbit tissues. Biochim Biophys Acta 1979;574:146-53.
[16] Sanders DJ, Minter HJ, Howes D, Hepburn PA. The safety evaluation of phytosterol esters. Part 6. The comparative absorption and tissue distribution of phytosterols in the rat. Food Chem Toxicol 2000;38: 485-91.
[17] Kritchevsky D, Tepper SA, Czarnecki SK, Wolfe B, Setchell KD. Serum and aortic levels of phytosterols in rabbits fed sitosterol or sitostanol ester preparations. Lipids 2003;38:1115-8.
[18] Connor WE, Lin DS, Pappu AS, Frohlich J, Gerhard G. Dietary sitostanol and campestanol: accumulation in the blood of humans with sitosterolemia and xanthomatosis and in rat tissues. Lipids 2005;40: 919-23.
[19] Jansen PJ, Lütjohann D, Abildayeva K, Vanmierlo T, Plosch T, Plat J, et al. Dietary plant sterols accumulate in the brain. Biochim Biophys Acta 2006;1761:445-53.
[20] Salen G, Horak I, Rothkopf M, Cohen JL, Speck J, Tont GS, et al. Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolemia with xanthomatosis. J Lipid Res 1985;26:1126-33.
[21] Heverin M, Bogdanovic N, Lutjohann D, Bayer T, Pikuleva I, Bretillon L, et al. Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. J Lipid Res 2003.
[22] Bhattacharyya AK, Connor WE. Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J Clin Invest 1974;53:1033-43.
[23] Mellies MJ, Ishikawa TT, Gluek CJ, Bove K, Morrison J. Phytosterols in aortic tissue in adults and infants. J Lab Clin Med 1976;88:914-21.
[24] Miettinen TA, Railo M, Lepantalo M, Gylling H. Plant sterols in serum and in atherosclerotic plaques of patients undergoing carotid endarterectomy. J Am Coll Cardiol 2005;45:1794-801.