Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 18 (2007) 580–586
Journal of Nutritional Biochemistry

RESEARCH ARTICLES

Phytosterols, but not pectin, added to a high-saturated-fat diet modify saturated fatty acid excretion in relation to chain length

Gemma Brufau[a], Miguel Angel Canela[b], Magda Rafecas[a,*]

[a]Department of Nutrition and Food Science-CeRTA, University of Barcelona, 08028 Barcelona, Spain
[b]Department of Applied Mathematics and Analysis, University of Barcelona, 08007 Barcelona, Spain

Received 25 July 2006; received in revised form 28 September 2006; accepted 6 October 2006

Abstract

The main objective of this article was to study how the excretion of saturated fatty acids (SFA) is modified after the consumption of a high-saturated-fat diet that was supplemented with phytosterol and pectin. We present the results of a longitudinal 4-week study on guinea pigs. Diets were supplemented with 0.33% of cholesterol and differed in the content of pectin (three levels) and of phytosterols (three levels). Seventy-two female Dunkin Hartley guinea pigs were randomly assigned to the treatment groups (8 animals/group). Addition of phytosterol resulted in a decrease of lauric (12:0) and myristic (14:0) excretions and in an increase of arachidic (20:0) and behenic (22:0) excretions. Palmitic (16:0) and stearic (18:0) acids did not show a clear change after phytosterol supplementation. Addition of pectin resulted in a decreased excretion of all SFA, although this was not significant. These results suggest that phytosterols added to a high-saturated-fat diet enhance the absorption of the most atherogenic fatty acids (lauric and myristic) after 1 week of treatment, as compared with the high-saturated-fat diet alone.

Keywords: Fatty acid chain length; Phytosterol; Pectin; SFA excretion; Saturated diet

1. Introduction

The marked hypercholesterolemic effect of saturated fatty acids (SFA) has long been known [1,2]. Recent investigations have shown that fatty acids may be classified into three metabolic groups. The first group is mainly made up of short-chain fatty acids such as butanoic (4:0), hexanoic (6:0), octanoic (8:0) and the medium-chain fatty acid decanoic (10:0), which are rapidly oxidized to acetyl CoA in the liver. These fatty acids do not alter the composition of the lipid pool in the liver, the concentration of free or esterified cholesterol in the hepatocyte or hepatic LDL receptor activity. Therefore, they are biologically neutral with respect to the regulation of the LDL cholesterol concentration. Interestingly, the long-chain SFA, stearic acid, also appears to belong to this biologically neutral group [3].

The second group includes lauric, myristic and palmitic acids, which inhibit LDL receptor activity [4], enhance LDL cholesterol production and increase the concentration of LDL cholesterol in the serum [3,5].

The third group includes the unsaturated fatty acids, which may be divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) *n*-6 and *n*-3. It has been reported that both MUFA and PUFA, when replaced by SFA, reduce total and LDL cholesterol concentrations [6], although the mechanisms underlying these effects are different. It has been suggested that MUFA, especially oleic acid, increase hepatic uptake of LDL particles as well as enhance the activity of cholesterol ester transfer protein [7]. *n*-6 PUFA reduce cholesterol levels by increasing the expression of hepatic LDL receptors and increasing the activity of 7α-hydrolase [8]. Finally, *n*-3 PUFA reduce blood cholesterol and triglyceride concentrations by reducing lipogenesis and secretion of VLDL, increasing lipoprotein lipase activity and enhancing cholesterol reverse transport [8,9].

The finding that SFA (lauric, myristic and palmitic acid) may have different metabolic effects on cholesterol levels or coronary heart disease (CHD) is relevant for practical dietary recommendations since those fatty acids that increase cholesterol levels contribute to the major portion of SFA intake in developed countries. In addition, there are epidemiological evidences that support the assumption that there is

* Corresponding author. Tel.: +34 934034841; fax: +34 934035931.
E-mail address: magdarafecas@ub.edu (M. Rafecas).

0955-2863/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2006.10.003

Table 1
Composition of diets fed to guinea pigs

Ingredients[a] (g/100 g)						
Pectin	Phytosterols[b]	Protein[c]	Fat[d]	Sugars[e]	Insoluble fiber[f]	Mineral and vitamin mix[g]
0	0	18.3	15.9	38.4	12.7	6.5
0	1.37	18.5	17.6	40.0	11.9	6.7
0	2.45	17.7	19.6	39.4	11.5	6.5
3.7	0	18.6	15.9	35.3	12.5	6.7
3.7	1.37	18.9	17.4	36.2	12.5	6.7
3.7	2.45	18.0	19.7	33.6	12.5	6.7
6.9	0	18.7	15.6	33.1	12.4	6.7
6.9	1.37	18.9	17.4	30.5	11.9	6.8
6.9	2.45	18.1	19.5	29.2	11.8	6.7

[a] All diets had been enriched with 0.33% of cholesterol.
[b] Added on top of the basal diet. The plant sterol ester mixture was mixed into the feeding and was composed of the following: 6.4% brassicasterol, 24.9% campesterol, 1.0% campestanol, 18.5% stigmasterol, 45.6% β-sitosterol, 1.8% sitostanol, 0.9% delta-5-avenasterol and 1.0% other sterols.
[c] Protein content was composed of casein.
[d] Fat content was composed of hydrogenated coconut oil.
[e] Sugar content was made up of corn starch, dextrose and sucrose.
[f] Insoluble fiber was made up of cellulose.
[g] Vitamin and mineral mix was adjusted to meet National Research Council requirements for guinea pigs. Detailed composition of the vitamin and mineral mix has been reported by Krause and Newton [37].

a positive correlation between SFA consumption and the incidence of CHD [10,11]. Therefore, general recommendations advise to limit the consumption of SFA to less than 10% of total daily energy supply [12], especially in Western countries where SFA consumption is very high [13,14].

Although a diet in accordance with the dietary reference intake should always be recommended, functional foods could be useful to further improve a favorable lipoprotein profile. During the past years, many studies have demonstrated the cholesterol-lowering effects of plant sterols and stanols [15–17] and of soluble fibers [18–20].

Plant sterols and stanols may displace cholesterol in the micelles since there is a competition between phytosterols/phytostanols and intestinal cholesterol with regard to incorporation into micelles. This effect will result in a decreased incorporation of cholesterol into micelles, a consequent reduced availability of cholesterol for absorption and lower serum LDL cholesterol concentrations [15–17].

Another food component with possible cholesterol-lowering properties is soluble fiber such as pectin. Pectin is able to bind bile acids, thus provoking a reduction in bile acid reabsorption by the small intestine [18–20].

Since plant sterols and pectin may both reduce plasma concentrations of total and LDL cholesterol, it may be interesting to study the effects of functional foods enriched with both compounds, especially since a great number of products enriched with them are in the food market. Functional foods enriched with phytosterols and pectin might have a synergic cholesterol-lowering effect, allowing for a reduction of the concentrations needed to achieve these effects separately.

Functional foods should be part of a healthy diet but should never be used to counterbalance the effects of an unhealthy diet. Thus, since the diet of many people in developed countries does not meet recommended intakes and since functional foods are extensively used, we consider that the effect of a high-saturated-fat diet supplemented with phytosterols and pectin should be studied.

Our group [21] has already reported that plant sterols may enhance lauric and myristic apparent absorption. In our opinion, however, it would be important to elucidate how this effect takes place. Thus, the aim of this article is to study how SFA excretion is modified during the consumption of a high-saturated-fat diet enriched with phytosterols and pectin for 4 weeks. For this study, guinea pigs were the animal of choice because their lipoprotein profile is similar to that of humans [22].

2. Materials and methods

2.1. Materials

All fatty acid methyl ester (FAME) standards with purity greater than 99% were purchased from Sigma Chemical Co. (St. Louis, MO). The identification of FAME was made with FAME Mix, C_4–C_{24} (Supelco, Bellefonte, PA).

Boron trifluoride in methanol (1.7 mol/L) and *n*-hexane were purchased from Merck (Darmstadt, Germany), and sodium chloride and anhydrous sodium sulfate were from Panreac (Barcelona, Spain).

Table 2
Content (in micrograms per milligram) of the main SFA in the diets

Pectin	Phytosterols	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$
PE0	PH0	54.61	20.62	11.35	12.63	0.18	0.05
PE0	PH1	41.98	15.88	9.35	10.00	0.18	0.06
PE0	PH2	47.26	17.87	10.53	11.38	0.26	0.10
PE1	PH0	59.61	22.23	12.12	13.30	0.19	0.07
PE1	PH1	44.82	16.73	9.66	10.13	0.19	0.07
PE1	PH2	48.04	18.12	11.04	11.31	0.26	0.11
PE2	PH0	60.76	22.52	12.41	13.46	0.19	0.05
PE2	PH1	44.32	16.75	9.99	10.44	0.19	0.07
PE2	PH2	45.34	17.19	10.30	10.98	0.25	0.10

Table 3
Variation of fecal SFA content (in micrograms per kilogram) and food consumption (in grams) during the course of the study

Week	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$	Consumption[a]
0	4.03	5.74	6.98	10.54	0.189	0.090	24.12
1	4.51	6.02	7.43	11.40	0.230	0.108	26.01
2	4.52	5.89	7.03	10.58	0.212	0.101	28.70
3	4.89	6.48	7.74	12.07	0.247	0.114	30.46
4	5.70	6.79	7.91	12.42	0.254	0.124	32.36

[a] Additional details about food consumption, feed efficacy and body weight of animals can be found in Ref. [21].

2.2. Diets

Nine isocaloric diets, designed to meet all the nutritional requirements for guinea pigs, were prepared by Mucedola SRL (Settimo Milanese, Italy), as it has been previously described [21]. Briefly, diets were supplemented with 0.33% of cholesterol, and their composition differed in the addition of pectin [three levels: PE0 (0%), PE1 (3.67%) and PE2 (6.93%)] and phytosterols [three levels: PH0 (0%), PH1 (1.37%) and PH2 (2.45%)] following a 3×3 factorial design. The chemical composition of the diets is shown in Table 1. Table 2 shows the content in diets (in micrograms per milligram) of the most important SFA found in diets and feces ($C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, $C_{20:0}$ and $C_{22:0}$).

2.3. Animals and analysis

Seventy-two female Dunkin Hartley guinea pigs, supplied by Harlan Interfauna Ibérica (Barcelona, Spain), weighing 300–350 g, were randomly assigned to the treatment groups (8 animals/group). The guinea pigs were housed two per cage in a light cycle room (lights on from 0800 to 2000 h), with free access to food and water. They were killed via heart puncture after administering halothane anesthesia. All procedures were approved by the Animal Care and Use Committee of the University of Barcelona. The duration of the treatments was 4 weeks, and the feces were collected three times every week and freeze-dried immediately.

The fatty acid content in feces and in diets was determined using the method of Lopez-Lopez et al. [23]. First, 25 μl of internal standard ($C_{21:0}$, 466 μg/ml) was added to 50 mg of freeze-dried fecal homogenate. Then, a small magnetic stirring bar was added, and the sample was saponified with 1 ml of sodium methylate (0.5 mol/L) and heated in a water bath at 90°C for 15 min. The tubes were removed from the water bath and cooled in a water bath at room temperature. Then, 1 ml of boron trifluoride–methanol (1.7 mol/L) was added. Next, they were placed in the water bath at 90°C for 15 min and subsequently cooled in the same manner as above, after which 400 μl of *n*-hexane was added. The tubes were shaken, and 1 ml of saturated solution of sodium chloride in distilled water was added, following centrifugation. The clear *n*-hexane top layer, containing the FAMEs, was transferred to another tube, adding a small quantity of anhydrous sodium sulfate. The tubes were stored at −20°C until injection into the gas chromatograph.

FAMEs were analyzed with an Agilent 4890D gas chromatograph, equipped with a flame ionization detector. The separation of FAME was done in a fused silica column (60 m×0.20 mm i.d., 0.2 μm). The split–splitless injector was used in split mode with a ratio of 1:30. Injector and detector temperatures were kept at 270°C and 300°C, respectively. Oven temperature was programmed as follows: an initial period of 5 min at 100°C, followed by an increase of 4°C/min up to 235°C and a final period of 2 min at this temperature.

Table 4
Mean SFA content (in μg/100 mg±S.D.) in feces

Pectin	Phytosterols		$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{20:0}$	$C_{22:0}$
PE0	PH0	Start	4.22±0.64	5.81±0.68	6.79±0.50	10.11±0.92	0.18±0.03	0.08±0.02
		End	8.20±1.44	9.91±2.51	1.24±3.19	15.10±5.16	0.24±0.08	0.12±0.03
PE0	PH1	Start	4.67±1.80	6.49±2.49	7.89±2.66	12.24±3.92	0.22±0.07	0.10±0.03
		End	5.56±1.37	6.28±1.33	7.94±1.94	12.55±3.23	0.26±0.06	0.13±0.03
PE0	PH2	Start	4.58±2.00	6.60±2.60	8.33±2.95	12.86±4.21	0.23±0.09	0.10±0.03
		End	5.88±1.86	6.37±1.91	8.07±2.57	13.99±5.36	0.34±0.13	0.16±0.05
PE1	PH0	Start	4.44±0.94	5.84±0.96	6.42±0.59	9.58±0.72	0.16±0.02	0.08±0.02
		End	6.80±2.82	8.38±2.89	9.14±2.63	13.05±4.98	0.22±0.07	0.11±0.04
PE1	PH1	Start	3.87±0.71	5.62±0.99	6.76±1.05	10.02±1.13	0.19±0.03	0.08±0.01
		End	4.48±0.44	5.46±0.84	6.86±1.34	10.61±2.79	0.22±0.05	0.11±0.02
PE1	PH2	Start	3.57±0.89	5.61±1.26	7.33±1.39	11.18±2.30	0.20±0.04	0.10±0.03
		End	4.49±1.73	5.27±1.78	7.32±1.80	12.88±3.51	0.34±0.08	0.17±0.04
PE2	PH0	Start	3.50±0.69	4.87±1.25	5.85±1.51	8.59±2.29	0.15±0.03	0.07±0.01
		End	3.64±1.84	7.59±0.84	7.94±0.84	11.03±1.50	0.18±0.03	0.09±0.02
PE2	PH1	Start	3.90±1.90	5.57±2.80	5.54±±2.75	10.02±3.63	0.18±0.07	0.09±0.03
		End	5.52±1.72	6.69±1.75	7.26±1.79	11.36±1.83	0.22±0.05	0.10±0.03
PE2	PH2	Start	3.79±0.85	5.50±1.46	6.89±1.68	1.09±2.37	0.18±0.03	0.10±0.01
		End	4.05±1.36	5.17±1.21	6.41±1.72	11.19±3.73	0.27±0.07	0.13±0.04

The number of cages in each group was four, with two animals per cage.

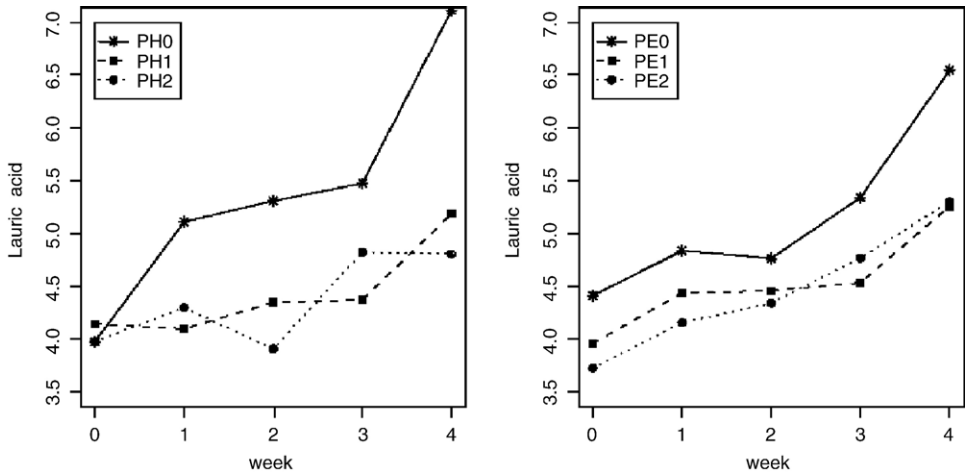

Fig. 1. Lauric acid content (μg/100 mg) in feces during 4 weeks of treatment after phytosterol (left) and pectin (right) intake.

Helium was used as the carrier gas. Chromatographic peaks were identified by comparing the retention times with those of known standards and by cochromatography. FAME quantification was performed by the internal standard addition method.

2.4. Statistical analysis

This article used a longitudinal analysis approach with five time points: Weeks 0, 1, 2, 3 and 4. The unit of analysis was the cage since feces were collected at this level. We have used the results at Week 0 as the baseline, subtracting them from the rest. This leaves us with four time points but allows us to control for the initial differences among the sample animals and for the fact that they received the same diet until Week 0, so that phytosterol and pectin effects cannot be estimated at this time. We have used the *xtreg* command of STATA 9.0 [24].

The analysis was based on a linear mixed-effects model, with the fecal content of the different SFA minus the baseline level as response variables and phytosterol (three levels) and pectin (three levels) as fixed effects. Since the SFA content of feces increases with time, we also included time effects in the model. Finally, we used food consumption per cage as a control variable to account for the possible influence of consumption on SFA fecal content. $P<.05$ was considered significant.

3. Results

Table 3 gives a summary of the variation of fecal SFA content during the course of the study, without distinction between treatments, which shows how the excretion varies depending on the week of treatment. It can be easily seen that there is an increase in fatty acid concentrations in the first week, a stabilization of these concentrations in the second week and a new increase in the last 2 weeks. We report lauric, myristic, palmitic, stearic, arachidic and behenic acids here.

Table 3 also includes food consumption (in grams) per cage, clearly showing linear time dependence (an increase of about 2 g/week). Due to a common time effect, SFA content and food consumption are positively correlated, but distinguishing the potential consumption effect on SFA

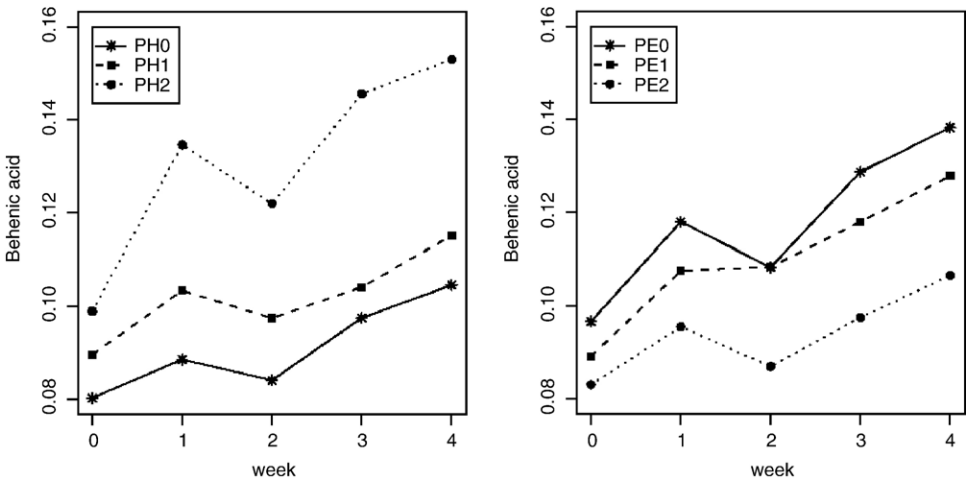

Fig. 2. Behenic acid (μg/100 mg) in feces during 4 weeks of treatment after phytosterol (left) and pectin (right) intake.

Table 5
Food consumption and final body weight (data were extracted from Ref. [21])

Pectin	Phytosterols	Food consumption (g/day/animal), mean±S.D.	Body weight (g), mean±S.D.
PE0	PH0	35.30±7.84	374.55±13.11
PE0	PH1	32.56±1.33	364.75±30.20
PE0	PH2	33.59±5.78	368.41±25.61
PE1	PH0	32.27±4.55	400.97±34.97
PE1	PH1	35.69±7.18	400.04±52.80
PE1	PH2	33.66±14.47	397.09±24.19
PE2	PH0	29.25±1.49	388.85±66.68
PE2	PH1	28.70±3.35	412.44±17.00
PE2	PH2	27.99±2.75	412.70±33.04

excretion from the time effect seems difficult. Nevertheless, we have included food consumption (also subtracting the baseline level at Week 0) as a control variable. We did not find a significant effect of phytosterol or pectin supplementation on consumption.

Table 4 shows the results of SFA at the beginning and at the end of the study. It can be easily seen that the supplementation effects are different depending on the chain length of SFA.

Fig. 1 shows the lauric acid content in feces during the 4 weeks of phytosterol (left panel) and pectin (right panel) treatments. In Fig. 2, the concentrations of behenic acid in feces during 4 weeks of treatment with phytosterol (left) and pectin (right) are shown.

Differences between the PH0 group and any of the other two phytosterol groups were highly significant ($P<.01$ in all cases) for lauric, myristic, palmitic and stearic acids. Differences between the PH2 group and the other two phytosterol groups (PH1 and PH0) were significant for arachidic ($P=.029$ and $P=.004$, respectively) and behenic ($P=.013$ and $P=.021$) acids. Comparing the data at the beginning of the study with those at the end, lauric acid levels increased by almost 78% in the control group but only by 20% in the PH2 group. Behenic acid level increased by 31% in the control group and around 55% in the PH2 group. According to the statistical analysis, the pectin effect was not significant for any of the SFA considered, although lauric and behenic acids had the same pattern of excretion, with the highest excretion found in the control group.

4. Discussion

Many studies have shown that a wide variety of functional foods enriched with plant sterols/stanols [15–17] and pectin [18–20] reduce cholesterol absorption. Daily intakes of 2 to 3 g of plant sterols and 6 to 12 g of pectin reduce serum LDL cholesterol concentrations by 10–15% and 5–16%, respectively. Nevertheless, most of these studies were performed using low-fat foods [15,25]. Furthermore, to our knowledge, no studies have been addressed to clarify how these compounds may counteract with the absorption of SFA, when added to a high-saturated-fat diet.

Although most of these studies were performed using low-fat diets, in Western countries, high-fat diets are commonly consumed [13,14]. Thus, to mimic the eating behavior of the Westerners, we decided to use high-saturated-fat diets for supplementations with pectin and plant sterols.

We have found differences in SFA excretion after phytosterol feeding, depending on the fatty acid chain length. Plant sterols enhanced the excretion of behenic and arachidic acids but decreased that of medium-chain SFA such as lauric and myristic acids. Another interesting result of our study was the way in which the fatty acids were excreted throughout the whole study. We found a similar pattern of excretion of all fatty acids in the control group (Figs. 1 and 2). The excretion increased after the first week, remained stable for a week and increased again in the last 2 weeks. However, phytosterol effect varies across SFA. The excretion of medium-chain SFA (lauric and myristic acids) increased by about 10% during the course of the study (Fig. 1), but that of long-chain SFA (behenic and arachidic acids) increased by more than 31%.

These differences in SFA excretion may be explained by the distribution of the lipid compounds in the mixed micelle. It is well known [26–30] that phytosterols can displace the compounds of the hydrophobic core of the micelles (such as hydrocarbon carotenoids), but no effect has been found on the compounds of the surface (such as tocopherols). Since lauric acid has 12 atoms of carbon, it is less fat-soluble and, thus, closer to the surface of the micelle than behenic acid (which has 22 atoms of carbon). Therefore, as it happens to carotenes, phytosterols may be more efficient in displacing behenic acid from the micelle than in displacing lauric acid.

Palmitic and stearic acids did not show a clear phytosterol effect. With chains from 16 to 18 atoms of carbon, they have a solubility between those of lauric and behenic acids. Thus, they may be situated between the core and the surface of the micelle.

Our results agree with those from a study of Normen et al. [30], who found that a saturated diet supplemented with phytosterols reduced SFA fecal excretion, this effect being more important for lauric and myristic acids than for palmitic and stearic acids. In addition, in the stanol group, they did not find such differences.

Pectin belongs to the family of soluble fibers, which are known to reduce cholesterol absorption in animal models [20,31] and in humans [19,32]. Since it is has already been proven in many studies that pectin with a high degree of methylation is needed to find hypolipidemic effects [20,33], we used pectin with a degree of methylation higher than 50% in our study. Nevertheless, we did not find differences in SFA excretion, although animals fed pectin showed lower concentrations in feces (nonsignificant). Moreover, excretion during the course of the study was between 30% and 40% in all groups. Several authors [34–36] have found higher concentrations of triglycerides in liver after pectin supplementation. These results might be in agreement with ours since one of the possible explanations is an enhanced

fatty acid absorption. This hypothesis was strongly supported in our previous paper [21], wherein we described that animals fed pectin had higher body weight and feed efficiency than those from the control group (Table 5). However, fatty acid excretion was not studied in any of the mentioned studies.

In conclusion, we suggest that plant sterols and pectin may have different effects on SFA excretion depending on the composition of the fat used in diets. Furthermore, the excretion of the most atherogenic fatty acids (lauric, myristic and palmitic) was higher after consumption of only high-saturated-fat diet than after consumption of this diet supplemented with phytosterols and pectin. Finally, we hypothesize that the mechanisms underlying these effects are different for pectin and phytosterols since the phytosterol effect is different depending on the chain length of the fatty acids, whereas that of pectin is not.

Acknowledgments

This study was financially supported by Programa Nacional de Recursos y Tecnologías Alimentarias (FIT-060000-20003-68). In addition, Gemma Brufau received a Fundació Pedro i Pons fellowship. Europastry provided the proximate analysis of the animal feeding.

References

[1] Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metabolism 1957;14:776–87.
[2] Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965;17:281–95.
[3] Kris-Etherton PM, Yu S. Individual fatty acid effects on plasma lipids and lipoproteins: human studies. Am J Clin Nutr 1997;65: 1628S–44S.
[4] Dietschy JM. Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. J Nutr 1998;128: 444S–8S.
[5] Temme EH, Mensink RP, Hornstra G. Comparison of the effects of diets enriched in lauric, palmitic, or oleic acids on serum lipids and lipoproteins in healthy women and men. Am J Clin Nutr 1996;63: 897–903.
[6] Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. Arterioscler Thromb 1992;12:911–9.
[7] Kurushima H, Hayashi K, Shingu T, Kuga Y, Ohtani H, Okura Y, et al. Opposite effects on cholesterol metabolism and their mechanisms induced by dietary oleic acid and palmitic acid in hamsters. Biochim Biophys Acta 1995;1258:251–6.
[8] Fernandez ML, West KL. Mechanisms by which dietary fatty acids modulate plasma lipids. J Nutr 2005;135:2075–8.
[9] Nestel PJ. Fish oil and cardiovascular disease: lipids and arterial function. Am J Clin Nutr 2000;71:2285–315.
[10] Dwyer J. Overview: dietary approaches for reducing cardiovascular disease risks. J Nutr 1995;125:656S–65S.
[11] Hu FB, Manson JE, Willet WC. Types of dietary fat and risk of coronary heart disease: a critical overview. J Am Coll Nutr 2001;20: 5–19.
[12] Krauss RM, Eckel RH, Howard B, Appel LJ, Daniels SR, Deckelbaum RJ, et al. AHA dietary guidelines. Revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000;102:2284–99.
[13] Kris-Etherton PM, Harris WS, Appel LJ, American Heart Association, Nutrition Committee LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747–57.
[14] Simopoulos AP. Omega-3 fatty acids in inflammation and autoimmune diseases. J Am Coll Nutr 2002;21:495–505.
[15] Mensink RP, Ebbing S, Lindhout M, Plat J, van Heutgen MM. Effects of plant stanol esters supplied in low-fat yoghurt on serum lipids and lipoproteins, non-cholesterol sterols and fat soluble antioxidant concentrations. Atherosclerosis 2002;160:205–13.
[16] Katan MB, Grundy SM, Jones P, Law M, Miettinen TA, Paoletti R, et al. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin Proc 2003;78:965–78.
[17] Miettinen TA, Gylling H. Plant stanol and sterol esters in prevention of cardiovascular diseases. Ann Med 2004;36:126–34.
[18] Galibois I, Desrosiers T, Guevin N, Lavigne C, Jacques H. Effects of dietary fibre mixtures on glucose and lipid metabolism and on mineral absorption in the rat. Ann Nutr Metab 1994;38:203–11.
[19] Brown L, Rosner B, Willett WW, Sacks FM. Cholesterol-lowering effects of dietary fiber: a meta-analysis. Am J Clin Nutr 1999;69: 30–42.
[20] Dongowski G, Lorenz A. Intestinal steroids in rats are influenced by the structural parameters of pectin. J Nutr Biochem 2004;15:16–205.
[21] Brufau G, Canela MA, Rafecas M. A high-saturated fat diet enriched with phytosterol and pectin affects the fatty acid profile in guinea pigs. Lipids 2006;41:159–68.
[22] Fernandez ML, Volek JS. Guinea pigs: a suitable animal model to study lipoprotein metabolism, atherosclerosis and inflammation. Nutr Metab 2006;3:17.
[23] Lopez-Lopez A, Castellote-Bargallo AI, Lopez-Sabater MC. Comparison of two direct methods for the determination of fatty acids in infant faeces. Anal Biochem 2000;282:250–5.
[24] StataCorp. Longitudinal/panel data reference manual. Stata Press, College Station; 2005.
[25] Garcia-Diez F, Garcia-Mediavilla V, Bayon JE, Gonzalez-Gallego J. Pectin feeding influences fecal bile acid excretion, hepatic bile acid and cholesterol synthesis and serum cholesterol in rats. J Nutr 1996;126:1766–71.
[26] Borel P, Grolier P, Armand M, Partier A, LaFont H, Lairon D, et al. Carotenoids in biological emulsions: solubility, surface-to-core distribution, and release from lipid droplets. J Lipid Res 1996;37: 250–61.
[27] Ntanios FY, Jones PJ. Dietary sitostanol reciprocally influences cholesterol absorption and biosynthesis in hamsters and rabbits. Atherosclerosis 1999;143:341–51.
[28] Ramjiganesh T, Roy S, McIntyre JC, Fernandez ML. The hypocholesterolaemic effects of sitostanol in the guinea pig are in part related to changes in hepatic lipids and lipoprotein composition. Br J Nutr 2001;85:165–72.
[29] Plat J, Mensink RP. Effects of diets enriched with two different plant stanol ester mixtures on plasma ubiquinol-10 and fat-soluble antioxidant concentrations. Metabolism 2001;50:520–9.
[30] Normen L, Ellegard L, Janssen HG, Steenbergen H, Trautwein E, Andersson H. Phytosterol and phytostanol esters are effectively hydrolysed in the gut and do not affect fat digestion in ileotomy subjects. Eur J Nutr 2006;45:165–70.
[31] Dongowski G, Lorenz A, Proll J. The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. J Nutr 2002;132:1935–44.
[32] Davidson MH, Dugan LD, Stocki J, Dicklin MR, Maki KC, Coletta F, et al. A low-viscosity soluble-fiber fruit juice supplement fails to lower cholesterol in hypercholesterolemic men and women. J Nutr 1998;128:1927–32.

[33] Furda I. Interaction of dietary fiber with lipids—mechanistic theories and their limitations. Adv Exp Med Biol 1990;270:67–82.
[34] Vergara-Jimenez M, Conde K, Erickson SK, Fernandez ML. Hypolipidemic mechanisms of pectin and psyllium in guinea pigs fed high fat-sucrose diets: alterations on hepatic cholesterol metabolism. J Lipid Res 1998;39:1455–65.
[35] Kreuzer M, Hanneken H, Wittmann M, Gerdemann MM, Machmuller A. Effects of different fibre sources and fat addition on cholesterol and cholesterol-related lipids in blood serum, bile and body tissues of growing pigs. J Anim Physiol Anim Nutr 2002;86:57–73.
[36] Kritchevsky D, Tepper SA. Influence of a fiber mixture on serum and liver lipids and on fecal fat excretion in rats. Nutr Res 2005;25:485–9.
[37] Krause BR, Newton RS. Animal models for the evaluation of inhibitors of HMG-CoA reductase. Adv Lipid Res 1991;1:57–72.