Nutrition, Metabolism & Cardiovascular Diseases (2011) xx, 1–6

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

Nutrition, Metabolism & Cardiovascular Diseases

Phytosterols supplementation decreases plasma small and dense LDL levels in metabolic syndrome patients on a westernized type diet

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Received 23 September 2010; received in revised form 9 December 2010; accepted 10 December 2010

KEYWORDS
Phytosterols;
Small and dense LDL levels;
Metabolic syndrome

Abstract *Background and aims:* Several studies have observed a hypocholesterolemic effect of plant sterols in hypercholesterolemic patients on a balanced diet. The aim of this study was to examine the effect of phytosterol supplementation on risk factors of coronary artery disease in metabolic syndrome patients on a Westernized type diet.
Methods and results: In a randomized placebo-controlled design 108 patients with metabolic syndrome were assigned to consume either 2 plant sterol-enriched yogurt mini drink which provided 4 g phytosterols per day, or a yogurt beverage without phytosterols (control). The duration of the study was 2 months and the patients in both groups followed their habitual westernized type diet and recording it on food diaries. Blood samples were drawn at baseline and after 2 months of intervention. After 2 months supplementation with phytosterols, a significant reduction in total cholesterol, LDL-cholesterol, small and dense LDL (sdLDL) levels, as well as, apoB and triglycerides concentrations were observed in the intervention group ($P < 0.05$) compared to the control group. In addition, phytosterol supplementation lowered serum total cholesterol by 15.9%, LDL-cholesterol by 20.3% and triglyceride levels by 19.1% ($P = 0.02$, $P < 0.001$ and $P < 0.001$, respectively), although the patients kept their habitual westernized type diet. No differences were observed in HDL cholesterol, apoA1, glucose, C-reactive protein, fibrinogen levels and blood pressure.

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0939-4753/$ - see front matter
doi:10.1016/j.numecd.2010.12.004

Please cite this article in press as: Sialvera TE, et al., Phytosterols supplementation decreases plasma small and dense LDL levels in metabolic syndrome patients on a westernized type diet, Nutrition, Metabolism & Cardiovascular Diseases (2011), doi:10.1016/j.numecd.2010.12.004

Conclusions: Phytosterol supplementation improves risk factors of coronary artery disease even if the diet is a westernized type.

Introduction

The metabolic syndrome has become a subject of great interest due to the clustering of several cardiovascular risk factors, elevated plasma levels triglycerides and glucose, low HDL cholesterol, raised blood pressure and obesity. Most people with this syndrome have an atherogenic lipid profile and insulin resistance, which classifies them as a high risk group for cardiovascular disease and type 2 diabetes and thus lifestyle changes are proposed [1]. The new ATPIII guidelines for the treatment of the metabolic syndrome recommended an increase in monounsaturated fatty acids (MUFA) intake, while there is also clear evidence that phytosterols have a hypocholesterolemic effect [2].

Although dyslipidaemia of the metabolic syndrome patients is characterized by elevated triglycerides and/or low HDL-cholesterol, lipid management should be steered, along with LDL-cholesterol goals, according to European guidelines on cardiovascular disease prevention in clinical practice. In terms of nutritional treatment, except for individualized dietetic recommendations, it was clearly suggested that phytosterols may help reduce plasma concentrations of LDL-cholesterol [3].

More recently the European Food Safety Authority (EFSA) approved a health claim for plant sterols, describing them as agents recommended and consumed only by people who want to lower their blood cholesterol. In line with EFSA, a clinically significant LDL-cholesterol effect of about 9% reduction is considered possible and it can be achieved by a daily intake of 2–2.4 g of phytosterols in an appropriate food (e.g. plant sterols added to fat-based foods and low-fat foods such as milk and yoghurt). However, it is suggested that the recommended amounts and patterns of consumption required to lower blood LDL-cholesterol can be achieved as part of a balanced diet [4–7]. Furthermore, little is known on whether phytosterols have an effect when the background diet is a westernized type, rich in total and saturated fat.

Therefore, the purpose of this study was to examine the effect of phytosterol supplementation on risk factors of coronary artery disease in metabolic syndrome patients, on a westernized type diet.

Methods

Subjects

One hundred and eight patients, men and women, aged 30–65 years were recruited from the Lipid Clinic of "Evangelismos" University Hospital, Athens and from the "Eurocliniki" Clinic, Athens, Greece. All the subjects had metabolic syndrome, as identified by the International Diabetes Federation (IDF) with 3 of 5 metabolic syndrome criteria. Specifically, they had triglycerides (TG) $>$ 150 mg/dl, HDL-C $<$ 40 mg/dl for men, HDL-C $<$ 50 mg/dl for women, blood pressure $>$ 130 and/or 85 mmHg, fasting glucose $>$ 100 mg/dl, waist circumference $>$ 94 cm for men and 88 cm for women [8]. Exclusion criteria included the presence of type 1 or 2 diabetes, cardiovascular disease, malignancy or any chronic disease that might influence inflammatory markers. We also excluded those who were taking multivitamins or anti-inflammatory drugs, those who were smoking $>$10 cigarettes per day and those who were consuming $>$40 g alcohol per day. Furthermore, during the previous 6 months participants should not have taken part in any weight-reduction program or other nutritional interventions or have practiced regular extreme physical activity. The study protocol was approved by the Ethics committee of "Harokopio University", Athens, Greece and all participants signed an informed consent form.

Study design

It was a parallel, single-blind, randomized and placebo-controlled intervention study. On enrollment, participants were assigned to either the intervention ($n = 53$) or the control ($n = 55$) groups randomly with the use of a sequence of binary numbers. At their first appointment with the dietitian, all participants were informed about the study and asked to keep a 3-d food diary. During each treatment the subjects followed their habitual, westernized type, *ad libitum* diets without any further consultation. The intervention group (29 men and 24 women) received daily 2 plant sterols enriched yogurt mini drinks (ELAIS-Unilever Hellas SA) which provided 4 g phytosterols per day and were instructed to consume them after their meals (lunch and dinner) and to follow *ad libitum* their westernized type diet. Plant sterols are found in everyday foods like fruits and vegetables, vegetable oils and nuts and grains, have a chemical structure similar to that of cholesterol and are primarily betasitosterol, campesterol, and stigmasterol. The sterols used in the supplement in the present study, are exclusively obtained from plant sources. Some of the sterols are obtained from vegetable oils, such as sunflower seed, rape seed or soy bean and some are obtained from tall oil which is an oil obtained from pine trees".

Each plant sterol-enriched yoghurt mini drink (100 g) provided them with 50 kcal, 3.2 g proteins, 5.6 g carbohydrates, 1.5 g fatty acids (0.2 g saturated fatty acids (SFA), 0.4 g monounsaturated fatty acids (MUFA), 0.9 g polyunsaturated fatty acids (PUFA), 0.1 g salt and 1.1 g dietary fiber. The control group (31 men and 24 women) received 2 placebo yogurt mini drinks without phytosterols per day and were instructed to maintain their dietary habits. The duration of dietary intervention was 2 months in both groups. Height, body weight, and waist circumference were measured in all participants at the beginning and at the end of the study and Body Mass Index (BMI) was calculated. The intervention group was closely supervised by a dietitian who made weekly phone calls and had biweekly appointments with participants. During these sessions body weight was measured to assure weight control (in both intervention and

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control participants), and they handed in their 3-d food diary (every second session).

Energy and nutrient intake

Both groups kept food diaries for 3 days (2 week and 1 weekend days) at the beginning, at the middle and at the end of the study. The analysis of the food diaries was carried out with the use of NUTRITIONIST V diet analysis software (version 2.1, 1999; First Databank, San Bruno, CA).

Blood sampling and laboratory methods

The volunteers attended the Department of Cardiology and Lipids at "Eurocliniki" and "Evangelismos" Hospital twice for blood collection, at the beginning and the end of the study. Venous blood samples were obtained at 08.00 h after a 12 h overnight fast. Immediately after blood collection, plasma was separated by centrifugation at 4000 rpm for 10 min at 4 °C within 1 h of blood collection in a bench centrifuge. Aliquots of plasma were transferred into eppendorf tubes and stored at −80 °C until analysis.

Serum total cholesterol, LDL-cholesterol, TG, and HDL-cholesterol concentrations were determined by enzymatic colorimetric assays with the use of a Cobas Integra 800 Analyzer (Roche, Switzerland). C-reactive protein (CRP), Apolipoprotein A-I (ApoA1) and Apolipoprotein B (Apo B) were assayed by immunoturbidimetric method (Cobas Integra 800, Roche, Switzerland). Plasma glucose concentration was measured enzymatically with hexokinase (Cobas Integra 800, Roche, Switzerland), whereas plasma fibrinogen concentration was measured by coagulation analyzer TCT (Siemens Healthcare Diagnostics, USA).

The assay for plasma small and dense LDL consisted of two steps and it was based on the technique of using well-characterized surfactants and enzymes that selectively react with certain groups of lipoproteins [9,10]. In the first step, non-sd LDL lipoproteins were decomposed by a surfactant and sphingomyelinase (SPC) that was reactive to those non-sd LDL lipoproteins. The cholesterol released from such non-sd LDL lipoproteins was then degraded to water and oxygen by the action of enzymes. Cholesterol ester was hydrolyzed by the cholesterol esterase (CE) and oxidized by the cholesterol oxidase (CO). Produced hydrogen peroxide was finally decomposed to water by catalase. In the second step, another surfactant released cholesterol only from sdLDL particles and was then subjected to the enzyme reactions. Plasma small and dense LDL levels were measured with the use of a Cobas Integra 400 (Roche, Switzerland) and the kit was s LDL − EX "SEIKEN" (Randox Laboratories Ltd, UK).

Statistical analysis

Normally distributed continuous variables are presented as mean ± standard deviation, skewed as median (1st, 3rd quartiles) and categorical variables as frequencies. The normality of continuous variables was tested graphically according to P−P and Q−Q plots. Comparisons between variables at baseline and 2 months after intervention were performed using paired *t*-test for normally distributed variables and Wilcoxon rank test was used for the remaining skewed data. Intervention and control group comparisons were tested using independent *t*-test and Mann−Whitney *U*-test for normally distributed variables and skewed data, respectively. *P*-value < 0.05 was considered as statistically significant. Prior statistical power analysis showed approximate 80% power while posterior analyses indicated that for some markers as, plasma glucose, CRP, blood pressure and BMI, the number of participants surpassed the needs for statistical power. All statistical analyses were performed using PASW Statistics 18 (SPSS Inc, Chicago, IL).

Results

Macro- and micro- nutrient intake of the participants was assessed at baseline and 8 weeks after start of the study period, from food diaries (Table 1). In addition, clinical (lipid profile and blood pressure) and anthropometric measurements of the participants (weight, height and waist circumference) took place at baseline and 2 months after the intervention period (Table 2). Participants didn't consume any type of alcohol in all study period.

Energy and nutrient intake

No significant differences were observed for most macro- and micro-nutrient intakes within the study period for all participants (*P* for all > 0.05), (Table 1). Energy and cholesterol intakes which seemed to increase after 2 months of study period in control and intervention group, correspondingly ($P = 0.01$ and $P < 0.001$, respectively). In addition, participants seemed to reduce consumption of vitamin A in both groups in these 2 months (*P* for all < 0.05). However, no significant differences were observed between groups, for all nutrient intakes included energy, cholesterol and vitamin A consumption (*P* for all > 0.05). Participants kept their habitual westernized type diet, with large proportion of their energy intake coming from fat and especially saturated fatty acids.

Serum lipids, small and dense LDL (sdLDL), blood pressure, and CRP

In the present study, plant sterol supplementation improved serum lipoprotein profile. In particular, within the intervention group (comparison between baseline levels and levels at the end of the intervention), significant reductions were observed in total cholesterol, LDL−cholesterol, sdLDL, TG and Apo B levels (*P* for all < 0.05) (Table 2). On the other hand, no significant differences were observed in these parameters within in the control group, except from LDL-cholesterol levels, which were also reduced. Most importantly, between group analysis showed that total-, LDL-cholesterol, sdLDL, apo B and TG levels improved in the intervention compared to the control group (*P* for all < 0.05). No significant differences were observed on serum HDL-cholesterol, Apo A1 and fibrinogen concentrations (*P* for all < 0.05). Differences were also found in BMI, CRP, and glucose levels as well as in systolic and diastolic blood pressure, but these differences are of no clinical significance since the actual levels are really

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unchanged and they may have been observed due to the large sample size used in this analysis for these parameters. Finally, compared to the control group, supplementation of phytosterols for 8 weeks lowered serum total-, LDL-cholesterol and triglycerides levels by 15.9% ($P = 0.02$), 20.3% ($P < 0.01$), and 19.1% ($P < 0.001$) respectively.

Discussion

To the best of our knowledge, this is the first study which observed a decrease in sdLDL following phytosterol supplementation. Compared to the control group, consumption of plant sterols also lowered serum total cholesterol by 15.9%, LDL-cholesterol by 20.3%, and TG levels by 19.1%. It is very interesting to point out that the magnitude of this lowering effect was large and it was observed even though the patients followed their habitual, westernized type diet (high-fat, high saturated fat diet). The duration and the level of plant sterol supplementation may have affected this large cholesterol-lowering action, although there are studies indicating that the lowering effect of phytosterols on cholesterol levels reaches a plateau at approximately 2–2.5 g of supplementation [6]. In the present study, phytosterols were consumed as a double daily dose following each main meal (lunch and dinner), in contrast with a single afternoon dose given in other studies [11].

In agreement with our findings, several studies have already shown that the regular consumption of foods containing plant sterols reduce total cholesterol, LDL-cholesterol and Apo B levels [12]. Meta-analyses showed reductions of about 9% with 2 g/d phytosterols consumption, while HDL-cholesterol remained unchanged [5–7]. Low density lipoprotein cholesterol-lowering has been found to be greater (up to 15%–20%), if compliance is maintained or additional dietary modification is implemented, for example in a low-fat, equilibrated diet [2,3]. More recently, it has been reported that independent of the type of diet, the consumption of 3.3 g plant sterols per day reduced plasma concentrations of total, LDL-cholesterol and TC/HDL-cholesterol by 9.0, 12.4 and 9.6%, respectively [13]. In contrast, Ooi and co-workers did not observe any effect on lipid profile in patients with metabolic syndrome following phytosterol supplementation [14]. It has to be noted however, that this intervention lasted only 4 weeks, the level of supplementation was 2 g instead of 4 g per day which was the level in our study, and the baseline total cholesterol levels in these volunteers were lower than the ones observed in our study.

Contrary to previous research that shows a rather minimal effect of plant sterols on serum TG levels, we found 19% reduction in metabolic syndrome patients. In line with our findings, few more studies have reported a decrease on TG concentration by 9 up to 14% after plant sterol supplementation in hypercholesterolemic adults on doses of 1.6 g up to 3 g/day [15–17]. Probably, the changes in TG levels depend on the baseline values, that is, TG concentration decreases in participants with high baseline TG levels, as suggested by Derdemezis et al. [12]. Though, the subjects in the present study were metabolic syndrome patients with moderate hypertriglycerideamia.

It is widely known that dietary interventions can reduce LDL-cholesterol by either decreasing LDL particle number, particle size, or both [18]. To the best of our knowledge, there are only 3 research papers referring to the effects of phytosterols on LDL density. In both cases and in contrast to our study, no differences were observed following phytosterol supplementation. In particular, Matvienko et al fed subjects with phytosterol-supplemented ground beef (2.7 g/day) and did not observe a significant shift from

Table 1 Macro- and micro- nutrient intake of the participants at baseline and 2 months after the intervention.[a]

	Intervention group ($n = 53$)			Control group ($n = 55$)			P^c between group-comparisons
	Baseline	2 mo after intervention	P^b	Baseline	2 mo after intervention	P^b	
Total energy (Kcal/d)	2124 (1530, 2634)	2131 (1538, 259)	0.29	2042 (1538, 2702)	2134 (1811, 2746)	0.01	0.41
% Energy from							
Protein	16 (14, 19)	16 (14, 19)	0.48	16 (14, 17)	16 (14, 19)	0.80	0.58
Carbohydrates	36 (32, 44)	37 (32, 42)	0.39	36 (32, 42)	39 (35, 42)	0.27	0.16
Total fat	41 (34, 50)	44 (38, 51)	0.15	47 (36, 52)	43 (39, 51)	0.91	0.22
SFAs	15 (11, 17)	16 (13, 18)	0.29	17 (12, 18)	16 (13, 18)	0.99	0.44
MUFAs	15 (11, 18)	15 (12, 19)	0.31	16 (12, 18)	15 (12, 19)	0.68	0.59
PUFAs	3 (3, 6)	3 (3, 7)	0.20	3 (3, 6)	5 (3, 9)	0.00	0.18
Fiber (g/d)	12 (11, 19)	13 (10, 20)	0.32	16 (11, 21)	15 (11, 19)	0.66	0.27
Vitamin C (mg/d)	122 (52, 158)	122 (52, 158)	0.22	122 (53, 148)	122 (53, 148)	0.26	0.89
Vitamin E – Alpha Tocopherol (mg/d)	6 (5, 9)	7 (5, 10)	0.27	7 (5, 9)	8 (5, 10)	0.20	0.90
Vitamin A (RE)	503 (314, 689)	381 (127, 586)	0.03	537 (325, 675)	312 (167, 452)	< 0.001	0.10
Cholesterol (mg/d)	311 (220,399)	329 (288, 429)	< 0.001	332 (215,369)	312 (281, 373)	0.145	0.35

(SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids).
[a] Nutrient intakes are presented as median (25th percentile, 75th percentile) because their distributions were skewed.
[b] *P*-values were derived through comparisons between baseline and 2 months after intervention using Wilcoxon rank test.
[c] *P*-values were derived through comparisons between intervention and control group using Mann–Whitney *U*-test.

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larger to smaller LDL particles [19]. This suggests that the decrease observed in LDL-cholesterol levels was primarily due to a decrease in the number of LDL particles. Indeed, Nuclear Magnetic Resonance (NMR) analysis performed at week 4 showed that the subjects in the treatment group tended to have a lower mean LDL particle number than did the control subjects. In the second study, the objective was to investigate the effects of phytosterol consumption on several LDL electrophoretic characteristics and to compare, the effects of unesterified plant sterols and stanols on size and composition of LDL subclasses in hypercholesterolemic men and women [20]. The major finding of this study was also that the treatment with sterols and stanols did not affect LDL particle size phenotype in hypercholesterolemic subjects. Finally, in the third study, consumption of plant sterol and no-trans-fat margarines within recommended amounts although it reduced Apo B concentrations and the ability of HDL to accept lipids, no differences were observed on small and dense LDL-cholesterol concentrations [21]. Two more studies observed beneficial effects on small LDL concentrations [22,23]. However, the intervention was not designed to investigate the effects of phytosterols, solely, on the size of LDL particles. In particular, in a dietary portfolio intervention based on phytosterols, viscous fiber, vegetable protein and almonds a reduction in serum concentrations of all LDL fractions including small dense LDL was observed [22] and in another similar dietary portfolio intervention, a pronounced effect on LDL subclasses was also reported [23]. The fact therefore that in our study we noticed a decrease in TG and sdLDL levels could imply that neutral lipid transfer system may have been affected although HDL-cholesterol levels were not altered. Neutral lipid transfer system is responsible for the transfer of TG from very low density lipoproteins (VLDL) and chylomicrons to LDL and HDL and for the transfer of cholesterol esters in the opposite direction. A decrease in TG levels could mean a decrease in their transfer from VLDL to LDL and HDL. Hepatic lipase is an enzyme which is responsible for the hydrolysis of TG from LDL and the consequent production of smaller LDL particles, the so called sdLDL. Therefore, decrease in TG content of LDL could mean that hepatic lipase would not be able to act on this particle and as a consequence plasma content of sdLDL would decrease. In the same direction, at least a small decrease in HDL particle size could have been expected. Therefore, further research is necessary looking at the effects of phytosterols on Cholesterol Ester Transfer Protein (the enzyme responsible for these transfers) and HDL subclasses in metabolic syndrome patients.

There would be however the argument if a dietary modification would mimic the effects of phytosterols on plasma lipids. In our study, the decrease in total and LDL-cholesterol levels was quite significant and it is greater than what the current opinion is [4]. However, a change in the dietary habits toward a Mediterranean type dietary pattern (low in SFA, high in MUFA and PUFA, high in fruits and vegetables) could have an additional benefit in the lipid profile and the risk of cardiovascular diseases (CVD), since it has been found that the Mediterranean diet affects not only blood lipid levels but also endothelial function, blood pressure, and other risk factors of CVD [24,25].

In conclusion, the present study showed that a 2-mo dietary intervention with 4 g phytosterols per day,

Table 2 Clinical and anthropometric characteristics of the participants at baseline and 2 months after the intervention.[a]

	Intervention group ($n = 53$)			Control group ($n = 55$)			P^c between group-comparisons
	Baseline	2 mo after intervention	P^b	Baseline	2 mo after intervention	P^b	
Weight (kg)	91 ± 18	91 ± 18	0.01	87 ± 16	87 ± 16	0.23	0.01
BMI (kg/m^2)	30 (28, 34)	30 (28, 34)	0.03	28 (27, 31)	28 (27, 30)	0.18	< 0.001
Waist circumference (cm)	105 (97, 116)	104 (97, 115)	0.14	101 (93, 108)	100 (93, 107)	0.29	0.06
Total cholesterol (mg/dl)	239 ± 27	200 ± 25	< 0.001	216 ± 28	216 ± 23	0.98	< 0.001
LDL-cholesterol (mg/dl)	162 ± 22	128 ± 22	< 0.001	151 ± 23	149 ± 24	0.01	< 0.001
Small dense LDL (mg/dl)	45.7 (34, 53)	41.8 (30, 51)	0.02	39.7 (33, 50)	36.7 (31, 50)	0.48	0.01
HDL-cholesterol (mg/dl)	42 ± 9	42 ± 9	0.66	43 ± 9	43 ± 9	0.55	0.66
Triglycerides (mg/dl)	195 (159, 243)	149 (130, 205)	< 0.001	173 (156, 189)	170 (151, 197)	0.72	< 0.001
Apolipoprotein A1 (mg/dl)	136 ± 18	136 ± 18	0.66	139 ± 22	139 ± 22	0.67	0.66
Apolipoprotein B (mg/dl)	115 ± 24	107 ± 24	< 0.001	119 ± 23	119 ± 23	0.77	< 0.001
C-reactive protein (mg/L)	2 (2, 4)	2 (1, 4)	0.05	2 (1, 4)	2 (1, 4)	0.02	< 0.001
Fibrinogen (g/l)	3 (3, 4)	3 (3, 4)	0.38	4 (3, 5)	3 (3, 6)	0.10	0.06
Glucose (mg/dl)	101 (94, 112)	99 (92, 108)	0.05	100 (94, 101)	99 (92, 103)	0.96	0.03
Systolic BP (mm Hg)	135 (125, 140)	130 (120, 130)	< 0.001	130 (120, 130)	130 (120, 130)	0.18	< 0.001
Diastolic BP (mm Hg)	85 (80, 90)	85 (80,90)	< 0.001	85 (80, 90)	85 (80, 85)	0.41	0.01

(LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; BP, Blood Pressure).

[a] Normally distributed continuous variables are presented as mean ± standard deviation while skewed as median (25th percentile, 75th percentile).

[b] *P*-values were derived through comparisons between baseline and 2 months after intervention using paired *t*-test for normally distributed variables and Wilcoxon rank test for skewed.

[c] *P*-values were derived through comparisons between intervention and control group using independent *t*-test for normally distributed variables and Mann–Whitney *U*-test for skewed.

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improved lipid profile and resulted not only, to a decrease in LDL-cholesterol, but also to a decrease in sdLDL levels in metabolic syndrome patients. This beneficial effect was observed although the subjects were not asked to change their dietary habits. This is an important finding because it implies that phytosterols have a significant impact on the lipid profile even if the diet is still a westernized type (rich in total fat, and SFA, and low in MUFA, PUFA and dietary fiber). Therefore, a change in dietary habits toward a healthy dietary pattern, could have an additional benefit in the lipid profile and the risk of CVD.

Acknowledgments

The authors would like to thank all the participants of the study for their cooperation.

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Please cite this article in press as: Sialvera TE, et al., Phytosterols supplementation decreases plasma small and dense LDL levels in metabolic syndrome patients on a westernized type diet, Nutrition, Metabolism & Cardiovascular Diseases (2011), doi:10.1016/j.numecd.2010.12.004