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**Serum lipid responses to phytosterol-enriched milk in a moderate hypercholesterolemic population is not affected by apolipoprotein E polymorphism or diameter of LDL particles**

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Running title: Effects of phytosterols and Apo E genotype

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## ABSTRACT

**Background:** The importance of both LDLc size and the apolipoprotein E (Apo E) in the atherogenic process is known, but there is little information regarding the effect of phytosterols (PS) on these parameters.

**Objectives:** The aim of this study was to evaluate the influence of PS on lipid profile and LDLc size according to Apo E genotype.

**Subjects and methods:** This was a randomized parallel trial employing 75 mild-hypercholesterolemic subjects and consisting of two 3-month intervention phases. After 3 months receiving a standard healthy diet, subjects were divided into two intervention groups: a diet group (n=34) and a diet+PS group (n=41) that received 2 g/day of PS. Total cholesterol (TC), triacylglycerols, LDLc, HDLc, non-HDLc, apolipoproteins (Apo) A-I and B-100, LDLc size and Apo E genotype were determined.

**Results:** Patients receiving PS exhibited a significant decrease in TC (5.1%), LDLc (8.1%), non-HDLc (7.4%) and Apo B-100/Apo A-I ratio (7.7%), but these effects did not depend on Apo E genotype. No significant changes were found in lipid profile according to Apo E genotype when patients following dietary recommendations were considered as a whole population or separately. No variations in LDLc size were observed in any of the intervention groups.

**Conclusions:** The results of the present study show that Apo E genotype does not have an impact on the lipid response to PS as a cholesterol-lowering agent in mild-hypercholesterolemic patients. Furthermore, the evidence obtained confirms that LDLc particle size is not modified when PS are added to a standard healthy diet.

**Keywords:** phytosterols, apolipoprotein E, LDLc diameter, hyperlipidemia.

## INTRODUCTION

Phytosterols (PS) are known to reduce serum low-density lipoprotein cholesterol (LDLc) levels, and so food products containing these plant compounds are widely used as dietary therapy to reduce plasma cholesterol and the risk of atherosclerosis. The cholesterol-lowering action of PS is thought to occur, at least in part, through competition with dietary and biliary cholesterol for intestinal absorption in mixed micelle (Calpe-Berdiel *et al.*, 2009). The daily consumption of foods rich in PS has been shown to reduce the plasma concentration of LDLc by 10% (Katan *et al.*, 2003) without altering levels of high-density lipoprotein cholesterol (HDLc) or triacylglycerols (TAG) (Noakes *et al.*, 2005; Seppo *et al.*, 2007). PS in their free form are slightly soluble in fats; in fact, their solubility and hypolipidemic effects can be increased via esterification. Most previous studies have demonstrated that the dispersion of PS in different food forms substantially affects the extent to which levels of LDLc are reduced (Katan *et al.*, 2003). However, few studies have examined the hypocholesterolemic effect of PS supplementation via low-fat dairy beverages in the absence of lipid-lowering medication (Noakes *et al.*, 2005; Seppo *et al.*, 2007; Bañuls *et al.*, 2009). LDL particles are heterogeneous in terms of size, density and physical properties, and small dense LDL particles have recently been associated with an increased risk of coronary heart disease (CHD), even in the presence of a relatively normal LDLc concentration (St Pierre *et al.*, 2001). There is little and contradictory information regarding the effect of PS on LDLc size. The majority of data in the literature provide evidence that LDLc size does not vary when there is a decrease in total cholesterol (TC) and LDLc (Matvienko *et al.*, 2002; Charest *et al.*, 2005; Gignoux *et al.*, 2007), though some reports have shown that it increases in such conditions (St-Onge *et al.*, 2003; Varady *et al.*, 2005; Sheresta *et al.*, 2007).

Apolipoprotein E (Apo E) plays an essential role in the metabolism of both cholesterol—in which it takes part in cellular cholesterol efflux and reverse cholesterol transport—and TAG. Apo E is a major protein constituent of TAG-rich lipoproteins (chylomicron and VLDLc), particles and their remnants, and also of HDLc. It serves as a ligand for the uptake of these lipoprotein particles to their receptors (Mahley, 1988; Beisiegel, 1989). However, such functions are not uniformly effective, as Apo E is highly polymorphic, with three common alleles ( $\epsilon 2$ ,  $\epsilon 3$ ,  $\epsilon 4$ ) codifying its three main isoforms (E2, E3, E4). These proteins determine changes in Apo E plasma concentrations and differ in their affinity to their specific receptors. There are contradictory data about the response of Apo E phenotypes to diet and PS. Whereas some researchers have shown that subjects carrying the Apo E4 allele exhibit a more pronounced LDLc lowering than subjects carrying the Apo E3 or Apo E2 alleles when undergoing dietary fat and cholesterol restriction, other studies report no association between Apo E phenotype and lipid response to dietary intervention (Masson & McNeill, 2005; Ordovas, 2009). Similarly, the specific role of Apo E as a determinant of serum lipid response to PS remains controversial (Ishiwata *et al.*, 2002; Plat & Mensink, 2002; Sanchez-Muñiz *et al.*, 2009).

In this context, the purpose of the present study was to evaluate the potential modulation by the Apo E locus of serum lipid responses to low-fat milk enriched with PS and consumed as part of a standard healthy diet recommended by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) (Expert panel on the detection, evaluation and treatment of high blood cholesterol in adults, 2001) among individuals with mild-to-moderate primary hypercholesterolemia.

## SUBJECTS AND METHODS

### Subjects

Patients with untreated moderate hypercholesterolemia were recruited from the Service of Endocrinology and Nutrition of University Hospital Dr Peset (Valencia, Spain). Those between the ages of 18 and 76 years (inclusive) were eligible for inclusion in the study. Further inclusion criteria were a serum LDLc cholesterol concentration of between 160-190 mg/dl in patients with less than 2 cardiovascular risk factors, 130-160 mg/dl in patients that presented 2 or more cardiovascular risk factors, and a TAG concentration of <400 mg/dl in all cases. Cardiovascular risk factors were defined as follows: age  $\geq 45$  years in men and  $\geq 55$  years in women, a smoking habit, hypertension ( $\geq 140/90$  mmHg), diabetes mellitus, HDLc concentration of <40 mg/dl, and a family history of cardiovascular disease. Exclusion criteria were pregnancy or lactation, change of oral contraceptives, severe disease, a history of cardiovascular or chronic inflammatory disease, hypersensitivity to milk proteins, and lipid-lowering medication. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee of the Hospital. Written informed consent was obtained from all patients.

### Study design

On the basis of the above mentioned criteria, 81 patients were included in the study that consisted of a randomised parallel trial of two 3-month intervention phases. All subjects were submitted to a 3-month run-in period of a standard healthy diet recommended by NCEP-ATP III (Expert panel on detection, evaluation and treatment of high blood cholesterol in adults, 2001) in order to stabilise dietary patterns prior to treatment. During the following 3 months, two intervention groups were evaluated: an experimental group, whose members consumed 2 g of PS/day in 500 ml of low-fat milk

(diet plus PS group), and a control group that followed the dietetic guidelines of the NCEP-ATP III (diet group) and whose diet included 500 ml/day of standard low-fat milk.

A clinical examination was performed during the first visit (selection and inclusion), after the stabilization period (three months) and at the end of the trial (six months).

Participants received dietary guidance throughout the trial from an experienced dietician and detailed written and oral instructions concerning the precise amounts of food to be eaten and the quality of food, according to the main food groups. The recommended daily diet included TC (<200 mg), saturated fat ( $\leq 7\%$  of daily total energy), simple sugar content (<10%), enhanced consumption of monounsaturated fatty acids mainly from olive oil (up to 20% of total energy),  $\omega$ -3 fatty acids from fish, 2-3 pieces of fruit and unlimited vegetables. With respect to daily energy intake, 8372 kJ (2000 kcal) were proposed for men and 7116.2 kJ (1700 kcal) for women containing 18-19% proteins, 52-53% carbohydrates, 29-30% fats of total energy and 20-30 g of dietary fibre.

Adherence to the diet was monitored by means of 3-day food records (compiled on weekdays) and 24-hour diet recall at baseline, three months and six months (carried out during appointments with the dietician). Food intake was converted into energy and nutrients with the help of the Spanish Food Composition Table (Mataix *et al.*, 2003).

The composition database was created with AYS44 Diet Analysis software obtained from ASDE, SA (Valencia, Spain). Subjects were encouraged to maintain their normal pattern of activity.

The PS-enriched milk was produced by Unilever (Spain) and packed in white containers. In addition to 0.4 g of vegetal sterols, every 100 ml of milk provided 3.2 g protein, 4.7 g carbohydrates, 1.8 g fat (0.25 g saturated, 0.50 g monounsaturated, 1.05 g polyunsaturated) and 48 Kcal. The PS consisted of vegetable oil-based sterols esterified

with sunflower oil fatty acids, and contained  $\beta$ -sitosterol (70%), campesterol (15%) and  $\beta$ -sitostanol (10%). The control group received a commercially available low-fat milk with similar macronutrient composition and energy intake to that consumed by the PS group, but which did not include vegetal sterols. Subjects were recommended to consume the milk twice per day with meals.

Compliance was evaluated by interviewing the subjects and counting the unopened and unconsumed product packages returned to the clinic, and was recorded as the percentage of the scheduled servings consumed. Non-compliance was defined as the consumption of <80% of the servings scheduled to be consumed during the study period.

#### Blood sampling

Venous blood samples were collected from subjects after 12h overnight fasting at baseline and at three and six months. TC and TAG were measured by means of enzymatic assays (Bucolo G & David H., 1973; Allain et al., 1974), and HDLc concentrations were recorded using a direct method (Sugiuchi et al., 1995) with a Beckman LX-20 autoanalyzer (Beckman Coulter, La Brea, CA, USA). The intraserial variation coefficient was <3.5% for all the determinations. When TAG values were under 300 mg/dl, LDLc concentration was calculated using the Friedewald method (Friedewald *et al.*, 1972). Non-HDLc concentration was obtained by calculating the difference between TC and HDLc. Apolipoprotein A-I (Apo A-I) and apolipoprotein B-100 (Apo B-100) were determined by immunonephelometry (Dade Behring BNII, Marburg, Germany), with an intra-assay variation coefficient of <5.5%.

LDLc size was evaluated after electrophoresis in polyacrylamide gradient-gel electrophoresis (2-16%), using the method described by Nichols *et al.* (Nichols *et al.*, 1986).

To evaluate the effect of Apo E on their response to treatment, subjects were categorized into 3 groups -E2: E2/E2, E2/E3 and E2/E4; E3: E3/E3 and E4: E3/E4 and E4/E4-.

DNA was extracted for genotyping according to the method of Miller *et al.* (Miller *et al.*, 1988), and was stored at -80°C until analysis. Apo E was identified after PCR amplification of a 244 bp fragment using forward 5# -

ACAGAATTCGCCCCGGCCTGGTACAC-3# and reverse 5#-

TAAGCTTGGCACGGCTGTCCAAGGA-3# as oligonucleotides. PCR reaction was performed in a 25 µl total volume containing 0.3 mM of each primer, 0.2 mM of each dNTP, 1x Taq buffer, 2 mM MgCl<sub>2</sub>, 100 ng of genomic DNA and 2.5 U Taq polymerase (Netzyme) using a Master-Cycler thermocycler (Eppendorf Scientific, Inc., Westbury, NY, USA). Each reaction mixture was heated at 94°C for 30 s, 64°C for 30 s, and 72°C for 35 s. Forty cycles were performed. A final extension was carried out at 72°C for 5 min. The 8 µl PCR products were subjected to restriction enzyme analysis by digestion for 4 hours at 37°C with 0.4 U of the restriction endonuclease Hha I. The fragment products were then separated by polyacrylamide gel electrophoresis (8%). After electrophoresis, the gel was treated with silver staining and DNA fragments were visualized.

#### Statistical analysis

Statistical analyses were conducted using SPSS 15.0 software. Data are expressed as mean ± SD (for tables) or SEM (for figures). Baseline characteristics among the groups were analyzed by one way analysis of variance (ANOVA). Between-group and within-group differences were analyzed using a 2-factor repeated-measures ANOVA followed by a paired Student's test, and percentage of change in lipid parameters was analyzed by unpaired Student t-test. p<0.05 was considered significant.

## RESULTS

Of the 81 patients initially enrolled in the study, 6 dropped out for personal reasons, including lack of time and difficulties in attending the research clinic. Details regarding diet composition has been published previously (Hernández-Mijares *et al.*, 2010).

The baseline characteristics of participants according to their Apo E alleles are shown in table 1. No differences in anthropometrical characteristics or lipoprotein profile were found in relation to any of the parameters studied. At the end of the three month intervention period, patients on the healthy diet supplemented with PS exhibited a significant reduction in TC, LDLc, non-HDLc and Apo B-100/Apo A-I ratio when compared to baseline conditions. Conversely, these lipid parameters were unaltered in patients undergoing dietary therapy only. No changes were found with respect to HDLc, TAG (all values were <300 mg/dl) or LDLc size in any of the groups (Table 2). When patients were analyzed according to their Apo E alleles, we found that the effect of PS on lipid profile was independent of Apo E phenotype, with similar significant reductions being observed in TC, LDLc, non-HDLc and Apo B-100/Apo A-I ratio in patients with E3 or E4 phenotypes. Data related to the genotype E2 are not represented due to the negligible number of this type of patient in our study population (2 subjects in the group that followed a healthy diet and 4 subjects in the group receiving combined therapy).

The efficacy of PS, independent of Apo E polymorphism, is also represented in figure 1. No differences were observed between patients with Apo E3 phenotype and those with Apo E4 phenotype in the percentage of decrease in TC, LDLc, non-HDLc or Apo B/Apo A-I after addition of PS to the standard healthy diet.

## DISCUSSION

The results of the present study suggest that the Apo E genotype does not have an impact on lipid response to PS as a cholesterol-lowering agent in mild-hypercholesterolemic patients. Furthermore, we show that LDLc particle size is not modified when PS are added to a healthy diet.

The first step towards reducing LDLc in individuals with mild to moderate hypercholesterolemia is a modification of lifestyle and diet, in particular a reduction of intake of total saturated fat (Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults, 2001). In the present study, no change occurred in any of the lipoprotein profile parameters evaluated (including TC or LDLc) after three months following a healthy diet. At first sight, these results may be surprising; however, it should be taken into account that patients were submitted to a 3-month run-in period of a standard healthy diet in order to stabilise dietary patterns prior to intervention. Thus, a positive effect of these preparatory dietary measures is likely to have taken place before the intervention period of this trial began, which is in the line with previous reports in which changes in TC and LDLc occurred only during the first weeks of a healthy diet (Bae *et al.*, 1991, Bañuls *et al.*, 2009). The Apo E polymorphism may influence the absorption of cholesterol from the intestine and, consequently, the response of serum cholesterol to a diet. However, our experimental design does not allow this aspect to be assessed, as we have disguised the possible effects of a healthy diet.

Although the use of different doses of PS is reported in the literature, the recommended dose for hypercholesterolemic patients is in the range of 1-2 g/day (Expert panel on detection, evaluation and treatment of high blood cholesterol in adults, 2001).

Importantly, and in line with the results of several studies published by our group and by other authors (Katan *et al.*, 2003; Bañuls *et al.*, 2009; Hernández-Mijares *et al.*,

2010), we have previously confirmed that the ingestion of approximately 2 g PS per day produces a 7.0-10.0 % reduction in LDLc levels. In fact, in the present study, we achieved a total reduction of 5.1% in TC, 8.1% in LDLc, 7.4% in non-HDLc and 7.7% in Apo B-100/Apo A-I ratio after adding PS to a standard diet. **Previous** data suggest that an increase in Apo B-100, Apo B-100/Apo A-I ratio and non-HDLc is an important risk factor for CHD, proving to be even more relevant than lipid concentration per se (Ridker *et al.*, 2005). In the present study, we have observed decreases of 7.4% and 5.9% in the Apo B-100/Apo A-I ratio and 6.3% and 8.3% in non-HDLc in Apo E3 and E4 patients after PS consumption, respectively, suggesting that there is a reduction in cardiovascular risk following intake of PS by patients with both phenotypes which is in the line with others (Escuriol *et al.*, 2010).

Numerous studies have demonstrated that the predominance of small and dense LDLc particles is associated with an increase of cardiovascular risk (Packard, 2006). In fact, small and dense LDLc particles exhibit a reduced affinity for the LDL receptor (Nigon *et al.*, 1991) and a marked susceptibility to oxidation, inducing alterations in endothelial function. Our results support the idea that a decrease in TC and LDLc does not have beneficial effects on LDLc size. They suggest that PS do not affect LDLc particle size as part of their mechanism of action, which is in accordance with those of previous studies (Matvienko *et al.*, 2002; Charest *et al.*, 2005; Gignoux *et al.*, 2007). It could be argued that this response is to be expected, since changes in TAG are known to be the main cause of the modification of the size of LDLc particles, and PS therapy does not alter TAG serum levels. The fact that others have described an increase in LDLc diameter following therapy combining PS with soluble fiber or culinary oils (St-Onge *et al.*, 2003; Sheresta *et al.*, 2007) or sterols with exercise (Varady *et al.*, 2005) suggest

that the positive effect on LDLc size is induced by other stimuli that modify TAG concentration more than PS.

Despite the fact that most previous studies confirm the hypolipidemic effect of PS, it is not so clear whether this effect is influenced or not by Apo E genotype. Several studies have investigated plasma lipid responses to PS supplementation in the context of the ApoE locus, and although most have shown that sterols and stanols lower LDLc irrespectively of ApoE alleles (Kempen *et al.*, 1991; Geelen *et al.*, 2002; Ishiwata *et al.*, 2002; Lottenberg *et al.*, 2002; Plat & Mensink, 2002; Tammi *et al.*, 2002), one recent report concluded that PS therapy was of little value for E4 subjects with hypercholesterolemia due to the lack of significant benefits in relation to TC and LDLc concentrations (Sánchez-Muñiz *et al.*, 2009). Our results are in accordance with those of previous studies in which PS therapy was not influenced by Apo E genotype. Among these studies, several were carried out in normocholesterolemic subjects (Kempen *et al.*, 1991; Geelen *et al.*, 2002; Ishiwata *et al.*, 2002; Plat & Mensink, 2002; Tammi *et al.*, 2002) and others were performed in a hypercholesterolemic population (Lottenberg *et al.*, 2002; Sánchez-Muñiz *et al.* 2009) with different doses and different types of PS (free sterols and stanols or stanol esters). In all cases, with the exception of the study by Ordovas' group, non-significant variations were observed between the lipoprotein responses of individuals according to their Apo E phenotype (Sánchez-Muñiz *et al.*, 2009).

In summary, our results confirm and extend the positive effect of a PS supplement when administered as part of dietary measures to treat moderately hypercholesterolemic patients. PS reduced the lipoprotein-mediated risk of cardiovascular disease, although this effect was not mediated by responses that were specific to either Apo E phenotype or reductions in the size of LDL particles.

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Table 1. Baseline characteristics of serum lipids and apolipoproteins according to Apo E phenotype.

	E2	E3	E4	All
Subjects (n)	6	47	22	75
Male/Female (n)	2/4	13/34	7/15	22/53
Age (years)	49.0 ± 12.3	50.1 ± 11.3	49.6 ± 13.7	49.9 ± 12.0
BMI (Kg/m <sup>2</sup> )	27.9 ± 4.6	28.5 ± 4.4	27.7 ± 6.2	28.2 ± 5.0
Waist-hip index	0.89 ± 0.12	0.88 ± 0.10	0.90 ± 0.12	0.88 ± 0.11
Systolic BP (mmHg)	127 ± 12	129 ± 15	131 ± 18	130 ± 15
Diastolic BP (mmHg)	80 ± 15	82 ± 10	80 ± 15	81 ± 13
Smokers (%)	25.0	30.3	33.3	29.5
TC (mg/dl)	237.5 ± 25.7	236.9 ± 30.6	237.0 ± 27.8	237.0 ± 29.0
LDLc (mg/dl)	165.6 ± 20.1	164.4 ± 25.9	166.3 ± 20.0	165.1 ± 23.6
HDLc (mg/dl)	49.2 ± 9.0	50.2 ± 14.3	49.0 ± 13.3	49.8 ± 13.5
Non-HDLc (mg/dl)	188.3 ± 24.2	186.8 ± 28.7	187.9 ± 26.4	187.2 ± 27.3
TAG (mg/dl)	113.7 ± 54.3	111.6 ± 61.9	107.9 ± 66.5	113.3 ± 61.9
LDLc size (nm)	26.25 ± 0.74	26.21 ± 0.75	26.06 ± 0.84	26.17 ± 0.76
Apo B-100/Apo A-I	0.713 ± 0.096	0.728 ± 0.166	0.827 ± 0.191	0.757 ± 0.174

Data are expressed as mean ± SD. Values of serum TAG concentrations were normalized using a log transformation. No differences among groups were found by one-way ANOVA.

Abbreviations: BMI: body mass index; BP: blood pressure; TC: total cholesterol; LDLc: LDL cholesterol; HDLc: HDL cholesterol; TAG: triacylglycerols; Apo: Apolipoprotein.

Table 2. Serum lipoprotein profile in diet and diet plus phytosterols groups classified by Apo E phenotype at baseline and at the end of the intervention.

	Diet group			Diet + PS group		
	E3	E4	All	E3	E4	All
n	23	9	34	24	13	41
TC (mg/dl)						
Baseline	239.7 ± 36.3	245.8 ± 25.7	240.7 ± 32.4	234.4 ± 24.7	230.9 ± 28.6	234.0 ± 26.1
End	232.7 ± 32.3	244.2 ± 24.4	236.1 ± 29.9	224.9 ± 26.3*	213.0 ± 27.2*	221.1 ± 27.0**
LDLc (mg/dl)						
Baseline	164.3 ± 30.6	175.9 ± 16.4	161.9 ± 38.9	164.6 ± 21.4	159.7 ± 20.0	163.7 ± 20.8
End	158.7 ± 30.4	176.8 ± 18.7	163.5 ± 25.8	150.9 ± 19.8**	145.3 ± 22.3*	149.1 ± 20.8***
HDLc (mg/dl)						
Baseline	49.8 ± 12.0	52.1 ± 14.0	50.0 ± 12.2	50.5 ± 16.4	46.9 ± 13.0	49.6 ± 14.7
End	50.1 ± 10.6	47.4 ± 7.0	49.4 ± 9.5	53.5 ± 15.6	46.0 ± 12.6	51.4 ± 14.5

Non-HDLc (mg/dl)						
Baseline	189.9 ± 34.7	193.7 ± 23.1	190.7 ± 30.5	183.9 ± 22.2	183.9 ± 28.6	184.5 ± 24.6
End	182.8 ± 30.3	196.8 ± 20.1	186.8 ± 27.3	171.4 ± 27.4*	167.0 ± 30.0*	169.7 ± 27.6***
TAG (mg/dl)						
Baseline	127.9 ± 72.5	108.8 ± 35.4	126.3 ± 64.8	96.5 ± 46.9	121.4 ± 78.1	103.8 ± 58.6
End	119.4 ± 71.4	100.0 ± 38.5	116.1 ± 64.1	102.6 ± 69.4	108.5 ± 67.7	102.7 ± 64.7
LDLc size (nm)						
Baseline	26.11 ± 0.63	26.07 ± 0.56	26.12 ± 0.60	26.20 ± 0.62	25.90 ± 0.88	26.10 ± 0.74
End	26.40 ± 1.01	26.26 ± 0.40	26.30 ± 0.92	26.22 ± 0.74	26.06 ± 1.48	26.18 ± 1.01
Apo B-100/Apo A-I						
Baseline	0.754 ± 0.163	0.864 ± 0.163	0.786 ± 0.163	0.702 ± 0.168	0.801 ± 0.211	0.733 ± 0.181
End	0.759 ± 0.174	0.817 ± 0.158	0.774 ± 0.167	0.644 ± 0.180**	0.744 ± 0.168*	0.669 ± 0.175***

Data are expressed as mean ± SD. Values of serum TAG concentrations were normalized using a log transformation. \* p<0.05, \*\*p<0.01, \*\*\*p<0.001 when compared using a paired Student's t-test for all subjects (n=34 and 41 in diet and diet+PS group, respectively), or a 2-factor measure repeated ANOVA (phenotype and time) followed by a paired Student t-test.

Abbreviations: PS: phytosterols; TC: total cholesterol; LDLc: LDL cholesterol; HDLc: HDL cholesterol; TAG: triacylglycerols; Apo: Apolipoprotein.

Figure 1. Percentage of change in lipid parameters after intervention period in subjects receiving diet plus phytosterols, classified by Apo E genotype.

Data are represented as mean and SEM of 41 subjects for diet plus phytosterols group (n=24 for E3 and n=13 for E4)

No differences between groups were found by unpaired Student t-test.

Abbreviations: TC: total cholesterol; LDLc: LDL cholesterol; HDLc: HDL cholesterol;

Apo B: Apolipoprotein B-100; Apo A: Apolipoprotein A-I

