Modulation of plasma triglycerides concentration by sterol-based treatment in children carrying different genes

Ismael San Mauro Martín, Sara Sanz Rojo, Elena Garicano Vilar, Luis Collado Yurrita[1], Javier Andrés Blumenfeld Olivares[2]

Research Centers in Nutrition and Health, Madrid, Spain, [1]Department of Medicine, Universidad Complutense de Madrid, Madrid, Spain, [2]Hospital El Escorial, San Lorenzo de El Escorial, Madrid, Spain

ABSTRACT

Background	:	**Dyslipidemias have increased during the last decades in children.**
Aim	:	**The objective of this study was to analyze the influence of different polymorphisms in plasma triglyceride levels of children following a dietary treatment with plant sterols.**
Design	:	**A randomized, double-blind, crossover, controlled clinical trial was carried out in 26 children (16 women).**
Materials and Methods	:	**Commercial milk, with 2.24 g sterols, was ingested daily during 3 weeks , and the same amount of skimmed milk without sterols, during the 3 week placebo phase. Both phases were separated by a washout period of 2 weeks. At the beginning and end of each phase, blood draws were performed.**
Results	:	**Apolipoprotein A5 Ser19Trp ($P = 0.002$), peroxisome proliferator-activated receptor-alpha L162V ($P = 0.003$), APOE APOE2/3/4 ($P = 0.012$), and APOE APOE2,3,4 ($P = 0.025$) show statistically significant differences between their haplotypes in plasma triglyceride levels. Other genes did not show statistically significant differences.**
Conclusions	:	**Further studies are needed to establish which genotype combinations would be the most protective against hypertriglyceridemia.**
Keywords	:	Cardiovascular disease, children, genetic, sterols, triglycerides

INTRODUCTION

Childhood is probably the most important period for the establishment of healthy habits. It is also crucial for the development of obesity, as well as a critical moment for many metabolic, clinical, and psychological changes that may affect their health later on.[1] Due to this, many public health campaigns are targeted to children.

With the increase of obesity and dyslipidemias in this age group, the concern about the role of triglycerides on cardiovascular health has risen.[2] Hypertriglyceridemia is related to different elements of the metabolic syndrome, such as obesity, hypertension, and insulin resistance.[3,4]

Obesity has been linked with alterations in insulin levels and insulin resistance which, in turn, affect lipid metabolism. This results in an increase of triglycerides and low-density lipoprotein cholesterol (LDL-c)[5] and lower levels of high-density lipoprotein cholesterol (HDL-c).[1] In Han children, triglycerides and nonHDL-c are better risk predictors for cardiovascular diseases (CVDs).[6] In relation to

Address for correspondence: Dr. Ismael San Mauro Martín, Research Centers in Nutrition and Health, 43 Paseo de la Habana Street, 28036, Madrid, Spain. E-mail: info@grupocinusa.es

hypertension, the ratio triglycerides/HDL-c is associated with hypertension in obese and non-obese children.[7] Triglycerides, as well as LDL-c and total cholesterol (TC) are related to lesions in the aorta and coronary arteries. Despite the lack of knowledge of the role of triglycerides on the development of atherosclerosis,[3] it is known that abnormal lipid levels during childhood are related with dyslipidemias in adulthood, and the onset and severity of atherosclerosis.[2]

Hypertriglyceridemia factors are both genetic and environmental. Several genes are related to lipid metabolism.[8] Apolipoprotein A5 (APOA5) is considered a risk factor for CVD,[9] with an increase of triglycerides and a lowering of HDL-c in boys, but not girls.[10] Naturally occurring variants of the APOA5 gene have been associated with increased triglyceride levels and have been found to confer risk for CVDs. Functional analyses *in vitro* suggest that-1131T>C variant has minimal effect on *APOA5* expression. The-1131T>C variant is also a strong predictor of hypertriglyceridemia[11] and increased CVD risk.[12] On the contrary, some studies did not observe differences between polymorphism 113G>T or 56C>G and plasmatic triglycerides concentrations.[13] Some of the methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms are also associated with a congenital heart failure risk[14] and CVD,[15] especially C677T genetic polymorphism.[16] The hepatic lipase gene (LIPC) is responsible for the hydrolysis of triglycerides.[17] Genetic studies and numerous epidemiologic studies have identified Lp (a) as a risk factor for atherosclerotic diseases such as coronary heart disease and stroke,[18] as it is related to LDL-c.[19] In addition, the genetic load may indirectly influence cardiovascular risk. The APOE play an essential role in the catabolism of lipoproteins.[20]

Environmental factors, such as physical activity and diet are helpful to reduce triglycerides when they are secondary to obesity.[3]

Lipid metabolism is a well-defined responder to dietary intakes and a classic biomarker of cardiovascular health. For this reason, circulating lipid levels have become the key to shaping nutritional recommendations for better management of CVD.[8]

Some cholesterol-related gene-diet interactions are confirmed.[8] Plant sterols/stanols are bioactive components with similar functions as that of cholesterol in mammals. They have been postulated as beneficial regulator agents for the control of CVD.[21-23] Daily consumption of phytosterol-enriched foods is widely used as a therapeutic option to improve lipid levels in plasma.[24] This has already been observed in studies that studied other lipids, such as LDL-c.[25]

Some early reports found moderately elevated plant sterol levels to be positively associated with vascular disease;[26] although, others suggested an inverse or lack of relationship between circulating plant sterols and cardiovascular risk.[27,28]

Plasma triglycerides can be reduced by an average of 6%–9% by two of plant sterols a day in hypertriglyceridemic patients; although, this evidence warrants further evaluation.[29] Future studies should be carried out with the aim of learning the increasing difference of dietary treatment data available in concordance with individual genomic profiles. Some studies, like the one performed by Clifton *et al.*[30] show that using milk as a carrier for plant sterols is beneficial.

Aim

The aim of this study was to analyze the influence of different polymorphisms on triglyceride levels in children following a dietary treatment with plant sterols.

MATERIALS AND METHODS

A randomized double-blind controlled clinical trial was designed. Participants were recruited at Hospital El Escorial in Madrid. Before the trial, the participants received instructions on the purpose of the study and their parents signed an informed consent form.

The plant sterols were ingested using *Naturcol* milk (supplied by *Corporación Alimentaria Peñasanta, S. A.,* Granada, Spain), available on the market throughout the whole study. Treatment and placebo skim milk were identical in sensorial and nutrient composition.

Blood samples were taken at the beginning and at the end of each phase. Over 3 weeks, the participants ingested two glasses of either type of milk daily. Each glass had a standard capacity of 350 mL. The participants in the treatment group had a daily consumption of 2.24 g of plant sterols, contained in 350 mL of skim milk; the placebo group drank the same quantity of skim milk without sterols. An 80% target was set as the minimum threshold for consumption. Milk was packaged without labeling to ensure neither the participants nor the researchers were aware of the type of milk and were only differentiated by the lid's color. Groups were randomly assigned, using random number tables.

Sample size

The sample size consisted of 58 participants: 22 male and 36 female with a mean age of 8.82 ± 2.28 yrs. The sample was recruited based on TC as the primary biomarker, according to the clinical practice guidelines by the Spanish Pediatrics Association (AEP).[31]

Inclusion and exclusion criteria

Inclusion criteria
Children, aged 5–12 years old, with a TC >170 mg/dL and/or LDL-c >110 mg/dL were included in the study.

Exclusion criteria

TC <170 mg/dL and/or LDL <110 mg/dL, puberal development at the beginning of the trial (Tanner Stage II); lactose intolerance, allergy to cow's milk proteins, galactosemia, celiac disease, any known chromosomopathies, and growth hormone therapy for short stature.

Clinical analyses

The samples for the analytical tests were extracted by medical staff after a 12-h fast at the Clinical Analysis Unit in the SCCUHM and El Escorial Hospital, and in the San Carlos Specialty Unit in San Lorenzo de El Escorial.

The blood extraction protocol of the laboratory was as follows: blood extraction was performed with a gel serum tube, using the technique "Blood collection" with an s-Monovette in aspiration.[32-34] After extraction, the samples were kept at 5℃ ± 3℃ until their arrival at the laboratory. The centrifugation settings were 20℃ ± 5℃, for 10 min, at 1200 g. Stability: 1 week at 5℃ ± 3℃. The Ultraviolet-Visible spectrophotometry technique was used.

The laboratory that performed the tests is in the same city of the study and has the required legal accreditations, certificates, and standards, ISO 9001:2008, and accreditation 511/LE2114 according to criteria included in the UNE-EN ISO 15189 Standard.

Genetic

Genomic analysis was performed to see if genetic polymorphisms could be associated with a higher or lower response to the plant sterol treatment for hypercholesterolemia. Of 58 participants, 26 were recruited for the genetic test: 10 male and 16 female with a mean age of 8.7 ± 2.06.

Polymorphisms APOA5 C56G Ser19Trp, Prothrombin G20210A, F5 Arg506Gln, MTHFR C677T, LIPC C-514T, LPA I4300M, peroxisome proliferator-activated receptor (PPAR)_alpha L162V, APOA5 1131T>C, APOE APOE2/3/4, and APOE APOE2,3,4 were studied.

Genomic DNA, for genotyping the SNP, was extracted from saliva samples and genotyping was conducted using the Biobank Axiom1 96-Array from Affymetrix. Genotype calling was performed with respect to Affymetrix's best practice guidelines, including analysis with SNPolisher, assuming a quality control rate of >0.97.[35,36]

The extraction and purification of DNA from saliva samples was carried out as follows: each DNA extraction was run on an agarose gel to ensure high-quality and high-molecular-weight. To ensure maximum purity of the extracted DNA (ratio >1.7), the OD260/280 ratio was analyzed, that is, the optical density of the extracted DNA at 260-nm and 280-nm wavelengths. All analyses were performed in duplicate to ensure maximum precision and reliability in their results.

Variables and study factors

An *ad hoc* questionnaire was designed for the study. The questionnaire and anthropometric study were performed by a single-trained researcher, ensuring the homogeneity and standardization of the uniformity criteria and methodology to follow. The study variables were established in terms of the proposed objectives: sex, age, clinical and pharmacological history, sleep quality, health habits, use of tobacco and alcohol, intestinal transit, food consumption frequency, and physical activity. In addition, each participant's weight, height, waist perimeter, body mass index (BMI), fat percentage, subcutaneous fat percentage, and lean body mass (kg) were measured. Weight, BMI, and body composition were determined by means of tetra-polar multi-frequency (20 and 100 kHz) electrical bioimpedance, In Body Model *270*, following the usual standard protocol and the manufacturer's recommendations. Waist perimeter was measured with a flexible nonelastic metal measuring tape with a range of 0.1 mm–150 cm. The analytical markers were as follows: lipid profile TC, HDL-c, LDL-c, and non-HDL-c. Confounding factors were also considered with an affinity table after ingestion (>95%), monitoring of the nonmodification of baseline habits during the trial, and a record of food consumption frequencies to control the ingestion of foods that may influence the metabolism of cholesterol upward or downward.

Statistical analysis

The data were analyzed using the SPSS 21.0 statistical package (IBM Corp., Armonk, NY: USA). A descriptive analysis was first made of the socio-demographic and anthropometric data, the baseline, and the final lipid values under the ingestion of *Naturcol* and the placebo. The normality of the lipid values was determined using the Shapiro–Wilk test. To analyze the efficacy of the ingestion of *Naturcol* and the placebo, the difference in lipid values was calculated before and after ingestion, also applying the Student's *t*-test, for related samples, or the Wilcoxon rank-sum test according to the compliance with the assumption of normality of the dependent variables. The efficacy of the intervention was verified by comparing the differences (final-baseline) in the ingestion of milk with sterols and the placebo, by applying the Student's *t*-test for related samples, or the Wilcoxon rank-sum test depending on the compliance with the assumption of normality of the lipid increase. The effect size and the proportion of the mean differences were calculated regarding the standard deviation of the baseline or milk with sterols. The level of significance applied was 5%.

The study was approved by the Bioethics Committee of Hospital El Escorial in Madrid. It followed the ethical principles enshrined in the Helsinki Declaration, the recommendations for good clinical practice, current

Spanish legislation regulating clinical research in humans, and the protection of personal and bioethical data (Royal Decree 561/1993 on clinical trials and 14/2007, July 3, for biomedical research).

RESULTS

A total of 26 participants (16 women and 10 men) completed the trial. They had an average age of 8.7 ± 2.06 years and weighed 69 kg (BMI 23.7 kg/m²) [Table 1]. Baseline TC was 236.6 mg/dL, LDL-c was 157.3 mg/dL and HDL-c 58.2 mg/dL. There were no differences between centers in volunteer demographics. In Table 2, the descriptive statistic of genes and haplotypes can be found.

PPAR-alpha CG, APOA5 1131T>C TC, APOE2/3/4 TC, and APOE2,3,4 TC showed the best decreases in this trial. Only APOA5 Ser19Trp (*P* = 0.002), PPAR-Alpha L162V (*P* = 0.003), APOE APOE2/3/4 (*P* = 0.012), and APOE APOE2,3,4 (*P* = 0.025) show statistical differences between their haplotypes. The biggest difference can be observed for APOA5 Ser19Trp, in which the CG haplotype shows an increase on TG levels with the ingestion of plant sterols. LPA I4300M also shows opposite results depending on the haplotype, but this difference is not as remarkable [Figure 1].

No statistically significant differences between the decrease percentage of triglycerides and the genotype were observed for the following genes: MTHFR C677T (*P* = 0.225), LIPC C-514T (*P* = 0.732), LPA I4300M (*P* = 0.127) and APOA5 1131T>C (*P* = 0.616). Prothrombin G20210A and F5 Arg506Gln genes could not be subjected to analytical study, because each gene had only one haplotype.

DISCUSSION

Important studies[37] evaluating the benefit of plant sterols consumption on cardiovascular risk biomarkers can be found, as a meta-analysis of more than 40 clinical trials.[38] Daily consumption of phytosterols-enriched foods is widely used as a therapeutic option to lower plasma cholesterol and atherosclerotic disease risk.[39]

Demonty *et al.* noted that triglyceride levels can be reduced by 6%–20% at intakes of 1.5–2 g/day of plant sterol/stanol, with essentially no effect on HDL-c.[40] Pooled analyses showed a modest reduction in plasma triglycerides of 6% and 4% for recommended intakes of plant sterols (1.6–2.5 g/day) or plant stanols (2 g/day), respectively.[41] Indeed, evidence suggests a relationship between baseline triglyceride levels and the magnitude of this effect, with 9% reduction when baseline triglycerides were 1.9 mmol/L (170 mg/dL), but no effect at baseline levels of 1.0 mmol/L (90 mg/dL).[40]

Regarding genetics, it has been observed that the ε4 allele of the ApoE4 is a major risk factor for coronary heart disease. However, the same results are not observed in the case of ApoE2, whose polymorphisms do not seem significantly related to coronary risk.[42]

In mice, overexpression of the human *APOA5* gene markedly decreases plasma TG concentration, whereas mice lacking the *Apoa5* gene become severely hypertriglyceridemic.[43,44] Maász *et al.*[45] suggest that the APOA5 C56G Ser19Trp allele can confer risk exclusively for the development of large-vessel associated stroke. Thereby, the 56G allele differs from the APOA5 T-1131C allelic variant, which has been previously identified as a risk factor for all subgroups of the stroke disease, namely, the C allele variant, which is a risk factor for heart disease and ischemic stroke[46,47] by increasing levels of triglycerides. This polymorphism has a significant association with coronary heart disease risk.[48]
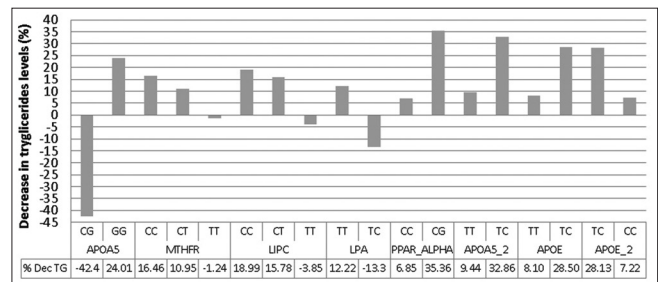

	CG	GG	CC	CT	TT	CC	CT	TT	TT	TC	CC	CG	TT	TC	TT	TC	TC	CC
	APOA5		MTHFR			LIPC			LPA		PPAR_ALPHA		APOA5_2		APOE		APOE_2	
% Dec TG	-42.4	24.01	16.46	10.95	-1.24	18.99	15.78	-3.85	12.22	-13.3	6.85	35.36	9.44	32.86	8.10	28.50	28.13	7.22

Figure 1: Percentage difference in triglyceride values with respect to before treatment, according to genes and haplotypes

Table 1: Descriptive statistic of anthropometric measurements and lipid profile

	Mean±SD		
	Total (*n*=26)	Males (*n*=10)	Females (*n*=16)
Age (years)	8.7±2.06	8.47±2.44	8.83±1.89
Weight (kg)	33.08±13.00	30.06±10.96	35.34±14.39
Height (m)	1.3±0.12	1.29±0.13	1.30±0.12
BMI (kg/m²)	18.79±4.20	17.21±3.18	19.98±4.59
Body fat (%)	25.49±9.66	20.84±6.99	28.97±10.16
Visceral fat (kg)	3.83±4.04	2.35±2.83	4.95±4.54
Muscle (kg)	11.55±4.46	11.08±4.1	11.91±4.86
Decrease (from baseline to final measures)			
Triglycerides (mg/dL)	13.90±37.57	4.26±61.29	15.60±19.81
Triglycerides (%)	5.4±30.0	6.8±29.1	5.2±31.5

SD: Standard deviation

Table 2: Descriptive statistic of genes and haplotypes

Gene	Haplotype	Frequency, *n* (%)
APOA5 C56G Ser19Trp (rs3135506)	CG	5 (19.2)
	GG	21 (80.8)
MTHFR C677T (rs1801133)	CC	3 (11.5)
	CT	6 (23.1)
	TT	13 (50)
LIPC C-514T (rs1800588)	CC	7 (26.9)
	CT	25 (96.2)
	TT	1 (3.8)
LPA I4300M (rs3798220)	TT	22 (84.6)
	TC	4 (15.4)
PPAR-alpha L162V (rs1800206)	CC	24 (92.3)
	CG	2 (7.7)
APOA5 1131T>C (rs662799)	TT	22 (84.6)
	TC	4 (15.4)
APOE Haplotype APOE2/3/4 (rs429358)	TT	5 (19.2)
	TC	21 (80.8)
APOE Haplotype APOE2,3,4 (rs7412)	TC	5 (19.2)
	CC	21 (80.8)

APOA5: Apolipoprotein A5, PPAR: Peroxisome proliferator-activated receptor, MTHFR: Methylenetetrahydrofolate reductase

The relationship between the LIPC and the influence on triglycerides has not been clearly established; although, it seems to be an inverse relationship type.[17] The influence that this gene has on the metabolism of glycerophospholipids[49] may also alter plasma concentrations thereof. Results published by Posadas-Sánchez *et al*.[50] suggested that the LIPC C-154T polymorphism is associated with cardiometabolic parameters and cardiovascular risk factors: under dominant model, the TT genotype was associated with increased levels of triglycerides/HDL-c index ($P = 0.046$). On the other hand, the same genotype was associated with the presence of small LDLs ($P = 0.003$). The risk analysis showed that under a dominant model, the LIPC C-514T polymorphism was associated with increased hypertriglyceridemia (odds ratio [OR] =1.36, $P = 0.006$) and coronary artery calcification (OR = 1.44, $P = 0.015$).[51]

Li *et al*. (2013)[48] showed that for the MTHFR C677T polymorphism, compared with wild CC genotype, heterozygosity CT increased the risk of congenital heart disease (OR = 2.249, 95% confidence interval [CI] 1.305–3.877, $P = 0.003$), the homozygous mutant genotype TT was associated with the risk of congenital heart disease significantly (OR = 3.121, 95% CI 1.612–6.043, $P = 0.001$).

Our results indicate that genes may have an impact in the effectiveness of plant sterol-based dietary treatments. Unfortunately, due to the heterogeneity of our sample, haplotypes were not distributed equally. It is the case of LIPC and LPA, which show interesting results on the graph, but with no statistical significance due to their small representation.

CONCLUSIONS

Our results indicate that carriers of APOA5, APOE APOE2/3/4, and APOE 2, 3, 4 genes may have different

outcomes, based on their haplotype, to the treatment with plant sterols for regulating hypertriglyceridemia. Even though other genes showed different results depending on the haplotype, they were not statistically significant.

Due to the importance of the treatment of dyslipidemias during childhood, more researches with bigger cohorts should be performed to understand the impact of nutrigenetics in the management of cardiovascular health in pediatric patients.

Limitations

The major limitation of this study was the sample size, which limited us from being able to extrapolate the results to the Spanish population. Due to the higher costs of more sophisticated analysis and a bigger cohort, we opted for smaller, humble research. Furthermore, the uneven size of the haplotypes groups may have interfered with our statistical analysis.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Romualdo MC, Nóbrega FJ, Escrivão MA. Insulin resistance in obese children and adolescents. J Pediatr (Rio J) 2014;90:600-7.

2. Margolis KL, Greenspan LC, Trower NK, Daley MF, Daniels SR, Lo JC, *et al*. Lipid screening in children and adolescents in community practice: 2007 to 2010. Circ Cardiovasc Qual Outcomes 2014;7:718-26.

3. Blackett PR, Wilson DP, McNeal CJ. Secondary hypertriglyceridemia in children and adolescents. J Clin Lipidol 2015;9:S29-40.

4. Costa GB, Horta N, Resende ZF, Souza G, de Barreto LM, Correia LH, *et al*. Body mass index has a good correlation with proatherosclerotic profile in children and adolescents. Arq Bras Cardiol 2009;93:261-7.

5. van Vliet M, van der Heyden JC, Diamant M, von Rosenstiel IA, Schindhelm RK, Heymans MW, *et al*. Overweight children with type 1 diabetes have a more favourable lipid profile than overweight non-diabetic children. Eur J Pediatr 2012;171:493-8.

6. Zhu WF, Liang L, Wang CL, Fu JF. Triglyceride and non-high-density lipoprotein cholesterol as predictors of cardiovascular disease risk factors in Chinese Han children. Indian Pediatr 2013;50:394-8.

7. Rodríguez-Morán M, Guerrero-Romero F, Aradillas-García C, Bermudez-Peña C, Simental-Mendia LE, Vargas Morales JM, *et al.* Atherogenic indices and prehypertension in obese and non-obese children. Diab Vasc Dis Res 2013;10:17-24.

8. Abdullah MM, Jones PJ, Eck PK. Nutrigenetics of cholesterol metabolism: Observational and dietary intervention studies in the postgenomic era. Nutr Rev 2015;73:523-43.

9. De França E, Silva DS, Silva TF, Dornelles CL, Alves JG, Alho CS, *et al.* APOA5 polymorphisms associated with lipid metabolism in brazilian children and adolescents. Genet Mol Res 2016;15:gmr.15017809. Doi: http://dx.doi.org/10.4238/gmr.15017809.

10. Guardiola M, Ribalta J, Gómez-Coronado D, Lasunción MA, de Oya M, Garcés C, *et al.* The apolipoprotein A5 (APOA5) gene predisposes caucasian children to elevated triglycerides and Vitamin E (Four provinces study). Atherosclerosis 2010;212:543-7.

11. Wang J, Ban MR, Kennedy BA, Anand S, Yusuf S, Huff MW, *et al.* APOA5 genetic variants are markers for classic hyperlipoproteinemia phenotypes and hypertriglyceridemia. Nat Clin Pract Cardiovasc Med 2008;5:730-7.

12. Triglyceride Coronary Disease Genetics Consortium and Emerging Risk Factors Collaboration, Sarwar N, Sandhu MS, Ricketts SL, Butterworth AS, Di Angelantonio E, *et al.* Triglyceride-mediated pathways and coronary disease: Collaborative analysis of 101 studies. Lancet 2010;375:1634-9.

13. San Mauro Martín I, Ruiz León AM, Garicano Vilar E, Blumenfeld JA, Pérez Arruche E, Arce Delgado E, *et al.* The relation between plasmatic triglycerides levels and polymorphisms -133T>C and 56C>G of ApoA5 codifying gene. JONNPR 2016;1:31-5.

14. Chen KH, Chen LL, Li WG, Fang Y, Huang GY. Maternal MTHFR C677T polymorphism and congenital heart defect risk in the Chinese Han population: A meta-analysis. Genet Mol Res 2013;12:6212-9.

15. Zhang MJ, Li JC, Yin YW, Li BH, Liu Y, Liao SQ, *et al.* Association of MTHFR C677T polymorphism and risk of cerebrovascular disease in Chinese population: An updated meta-analysis. J Neurol 2014;261:925-35.

16. Kumar A, Kumar P, Prasad M, Sagar R, Yadav AK, Pandit AK, *et al.* Association of C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR gene) with ischemic stroke: A meta-analysis. Neurol Res 2015;37:568-77.

17. Edmondson AC, Braund PS, Stylianou IM, Khera AV, Nelson CP, Wolfe ML, *et al.* Dense genotyping of candidate gene loci identifies variants associated with high-density lipoprotein cholesterol. Circ Cardiovasc Genet 2011;4:145-55.

18. Nordestgaard BG, Chapman MJ, Ray K, Borén J, Andreotti F, Watts GF, *et al.* Lipoprotein(a)as a cardiovascular risk factor: Current status. Eur Heart J 2010;31:2844-53.

19. Donnelly LA, van Zuydam NR, Zhou K, Tavendale R, Carr F, Maitland-van der Zee AH, *et al.* Robust association of the LPA locus with low-density lipoprotein cholesterol lowering response to statin treatment in a meta-analysis of 30 467 individuals from both randomized control trials and observational studies and association with coronary artery disease outcome during statin treatment. Pharmacogenet Genomics 2013;23:518-25.

20. San Mauro-Martín I, de la Calle-de la Rosa L, Sanz-Rojo S, Garicano-Vilar E, Ciudad-Cabañas MJ, Collado-Yurrita L. Genomic approach to cardiovascular disease. Nutr Hosp 2016;33:148-55.

21. San Mauro Martín I, Collado Yurrita L, Ciudad Cabañas MJ, Cuadrado Cenzual MÁ, Hernández Cabria M, Calle Purón ME, *et al.* Risk management of cardiovascular disease through milk enriched with sterols in a young-adult population; randomized controlled clinical trial. Nutr Hosp 2014;30:945-51.

22. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult treatment panel III) final report. Circulation 2002;106:3143-421.

23. Alonso Karlezi RA, Mata Pariente N, Mata López P. Control de las hiperlipemias en la práctica clínica. Rev Española Cardiol Supl 2006;6:24G-35G.

24. Matthan NR, Zhu L, Pencina M, D'Agostino RB, Schaefer EJ, Lichtenstein AH. Sex-specific differences in the predictive value of cholesterol homeostasis markers and 10-year cardiovascular disease event rate in Framingham Offspring Study participants. J Am Heart Assoc 2013;2:e005066. Doi: 10.1161/JAHA.112.005066.

25. San Mauro Martín I, Blumenfeld Olivares JA, Pérez Arruche E, Arce Delgado E, Ciudad Cabañas MJ, Garicano Vilar E, *et al.* Genomic influence in the prevention of cardiovascular diseases with a sterol-based treatment. Diseases 2018;6. pii: E24.

26. Sudhop T, Gottwald BM, von Bergmann K. Serum plant sterols as a potential risk factor for coronary heart disease. Metabolism 2002;51:1519-21.

27. Pinedo S, Vissers MN, von Bergmann K, Elharchaoui K, Lütjohann D, Luben R, *et al.* Plasma levels of plant sterols and the risk of coronary artery disease: The prospective EPIC-Norfolk population study. J Lipid Res 2007;48:139-44.

28. Escurriol V, Cofán M, Moreno-Iribas C, Larrañaga N, Martínez C, Navarro C, *et al.* Phytosterol plasma concentrations and coronary heart disease in the prospective Spanish EPIC cohort. J Lipid Res 2010;51:618-24.

29. Gylling H, Plat J, Turley S, Ginsberg HN, Ellegård L, Jessup W, *et al.* Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. Atherosclerosis 2014;232:346-60.

30. Clifton PM, Noakes M, Sullivan D, Erichsen N, Ross D, Annison G, *et al.* Cholesterol-lowering effects of plant sterol esters differ in milk, yoghurt, bread and cereal. Eur J Clin Nutr 2004;58:503-9.

31. Moráis López A, Lama More RA, Dalmau Serra J, Comité de Nutrición de la AE. Hypercholesterolemia:

A therapeutic approach. An Pediatr (Barc) 2009;70:488-96.

32. Lippi G, Avanzini P, Musa R, Sandei F, Aloe R, Cervellin G, *et al.* Evaluation of sample hemolysis in blood collected by S-monovette using vacuum or aspiration mode. Biochem Med (Zagreb) 2013;23:64-9.

33. Bowen RA, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. Biochem Med (Zagreb) 2014;24:31-44.

34. Sarstedt. Blood Collection with the S-Monovette®; 2018. Available from: https://www.sarstedt.com/fileadmin/user_upload/99_Gebrauchsanweisungen/Englisch_US_Code/644_c_PosterA3_AnleitungVenoeseBE_SafetyKanuele_GB_US_0314.pdf. [Last accessed on 2018 Mar 08].

35. Weale ME. Quality control for genome-wide association studies. Methods Mol Biol 2010;628:341-72.

36. Ziegler A. Genome-wide association studies: Quality control and population-based measures. Genet Epidemiol 2009;33 Suppl 1:S45-50.

37. Katan MB, Grundy SM, Jones P, Law M, Miettinen T, 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.

38. Abumweis SS, Barake R, Jones PJ. Plant sterols/stanols as cholesterol lowering agents: A meta-analysis of randomized controlled trials. Food Nutr Res 2008. DOI: 10.3402/fnr.v52i0.1811.

39. Rocha M, Banuls C, Bellod L, Jover A, Victor VM, Hernandez-Mijares A, *et al.* A review on the role of phytosterols: New insights into cardiovascular risk. Curr Pharm Des 2011;17:4061-75.

40. Demonty I, Ras RT, van der Knaap HC, Meijer L, Zock PL, Geleijnse JM, *et al.* The effect of plant sterols on serum triglyceride concentrations is dependent on baseline concentrations: A pooled analysis of 12 randomised controlled trials. Eur J Nutr 2013;52:153-60.

41. Naumann E, Plat J, Kester AD, Mensink RP. The baseline serum lipoprotein profile is related to plant stanol induced changes in serum lipoprotein cholesterol and triacylglycerol concentrations. J Am Coll Nutr 2008;27:117-26.

42. Zhang MD, Gu W, Qiao SB, Zhu EJ, Zhao QM, Lv SZ, *et al.* Apolipoprotein E gene polymorphism and risk for coronary heart disease in the Chinese population: A meta-analysis of 61 studies including 6634 cases and 6393 controls. PLoS One 2014;9:e95463.

43. van der Vliet HN, Schaap FG, Levels JH, Ottenhoff R, Looije N, Wesseling JG, *et al.* Adenoviral overexpression of apolipoprotein A-V reduces serum levels of triglycerides and cholesterol in mice. Biochem Biophys Res Commun 2002;295:1156-9.

44. Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, *et al.* An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. Science 2001;294:169-73.

45. Maász A, Kisfali P, Szolnoki Z, Hadarits F, Melegh B. Apolipoprotein A5 gene C56G variant confers risk for the development of large-vessel associated ischemic stroke. J Neurol 2008;255:649-54.

46. Pi Y, Zhang L, Yang Q, Li B, Guo L, Fang C, *et al.* Apolipoprotein A5 gene promoter region-1131T/C polymorphism is associated with risk of ischemic stroke and elevated triglyceride levels: A meta-analysis. Cerebrovasc Dis 2012;33:558-65.

47. Zhou J, Xu L, Huang RS, Huang Y, Le Y, Jiang D, *et al.* Apolipoprotein A5 gene variants and the risk of coronary heart disease: A casecontrol study and metaanalysis. Mol Med Rep 2013;8:1175-82.

48. Li YY, Wu XY, Xu J, Qian Y, Zhou CW, Wang B, *et al.* Apo A5 -1131T/C, FgB -455G/A, -148C/T, and CETP TaqIB gene polymorphisms and coronary artery disease in the Chinese population: A meta-analysis of 15,055 subjects. Mol Biol Rep 2013;40:1997-2014.

49. Demirkan A, van Duijn CM, Ugocsai P, Isaacs A, Pramstaller PP, Liebisch G, *et al.* Genome-wide association study identifies novel loci associated with circulating phospho- and sphingolipid concentrations. PLoS Genet 2012;8:e1002490.

50. Posadas-Sánchez R, Ocampo-Arcos WA, López-Uribe ÁR, Posadas-Romero C, Villarreal-Molina T, León EÁ, *et al.* Hepatic lipase (LIPC) C-514T gene polymorphism is associated with cardiometabolic parameters and cardiovascular risk factors but not with fatty liver in Mexican population. Exp Mol Pathol 2015;98:93-8.

51. Wang H, Zhang D, Ling J, Lu W, Zhang S, Zhu Y, *et al.* Gender specific effect of LIPC C-514T polymorphism on obesity and relationship with plasma lipid levels in Chinese children. J Cell Mol Med 2015;19:2296-306.