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A genetic variant of PPARA modulates cardiovascular risk biomarkers after milk consumption

Running head: PPARA rs135549 modulates response to milk consumption

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ABSTRACT

Objective: The association of dairy food consumption with the risk of developing cardiovascular disease (CVD) has been investigated in many studies, but results have often been contradictory. The aim of the present study was to determine whether genetic polymorphisms are associated with inter-individual variation in the response of CVD risk biomarker values after milk consumption.

Research Methods & Procedures: Fourteen single nucleotide polymorphisms (SNPs) in nine genes related to lipid metabolism were examined in 161 volunteers randomly allocated to consume either 500 mL/d of skimmed (S) or semi-skimmed (SS) milk for 1 year in addition to their usual diets. Total cholesterol/HDL cholesterol (TC/HDL) and LDL/HDL cholesterol ratios were used as biomarkers of CVD risk. Three-way repeated-measures ANOVA was used to examine the effect of time, treatment (S or SS) and genotype on these biomarkers.

Results: A TT genotype for the proliferator-activated receptor alpha polymorphism (PPARA rs135549 SNP) was significantly associated with a reduction in the TC/HDL and LDL/HDL ratios after 12 months of S milk intake (mean reduction -0.29, 95% CI -0.63 - 0.05 [$p=0.0015$] and -0.31 95% CI -0.58 - -0.03 [$p=0.0005$] respectively). However, no differences were observed after consuming either S or SS milk in the C allele carriers.

Conclusions: Saturated fatty acid consumption has long been linked to an increased risk of CVD; indeed, the consumption of saturated fat-free products is recommended as a means of reducing this risk. However, the present results suggest that many people might not benefit from such general recommendations. Genetic analysis of PPARA rs135549 might help identify which individuals are more likely to benefit from reducing the saturated fatty acid content of their diet.

Keywords: milk; nutrigenetics; PPARA; lipid profile; rs135549

INTRODUCTION

Cardiovascular disease (CVD), which is intimately associated with diet, is the leading cause of morbidity and mortality worldwide [1]. Saturated fats have long been thought to contribute towards CVD since they increase plasma cholesterol [2], a marker of increased risk of CVD [3]. Indeed, reducing or avoiding foods rich in saturated fat is recommended as a means of helping prevent CVD in general and high-risk populations [4, 5; 6].

However, more recent studies examining the link between dairy fat (approximately 70% of which is saturated) and CVD have returned contradictory results. For example, some large epidemiological studies have associated the consumption of high-fat dairy products with an increased risk of developing CVD, while no such association has been found with respect to the intake of low-fat dairy foods [7, 8]. Yet other reports indicate the consumption of the latter to reduce this risk [9, 10].

Moreover, a recent survey of publications in this area suggested a high consumption of any type of milk to be no more associated with an increased risk of CVD than a low consumption [11, 12; 13, 14]. Meta-analyses have also highlighted the inconsistency of the results obtained in different studies [14, 15, 16]. In addition, recent short-term intervention studies on CVD biomarkers have indicated that while whole milk increases LDL cholesterol it also increases HDL cholesterol, and therefore might not affect the total cholesterol/HDL cholesterol ratio [13]. The confusion regarding the effect of dairy saturated fat on CVD risk has nonetheless resulted in a reduction in milk consumption [17], even though milk contains many important nutrients, including protein, minerals and vitamins. Further, milk fat provides a source of energy and bioactive molecules, and is an important delivery medium for fat-soluble vitamins etc. [18]. In addition, the phospholipids in milk have been associated with anti-inflammatory and antimicrobial activities, and even the inhibition of cholesterol absorption [19].

Knowing whether it is better to maintain or reduce milk consumption therefore requires a better understanding of the effect of dairy fat consumption on CVD risk.

It has been proposed that gene/diet interactions modulate responses to dietary factors [20, 21; 22; 23], and thus, the effect of dairy product consumption on the risk of developing CVD might have a genetic component. A better understanding of gene/diet interactions might lead to the development of

personalized dietary recommendations, based on an individual's genotype, for preventing or delaying the onset of chronic diseases.

In an effort to reduce the confusion regarding dairy fat consumption and CVD risk, the present work explored whether genetic polymorphisms within candidate lipid metabolism genes are associated with inter-individual variation in terms of CVD risk biomarker values following the consumption of skimmed or semi-skimmed milk.

MATERIALS AND METHODS

Subjects and study design

A total of 161 volunteers aged between 25 and 65 years (135 men and 26 women) were randomly allocated to two parallel intervention groups. Both groups continued with their regular diets, but the members of one consumed 500 ml/day of semi-skimmed milk (SS; n=85) while those of the other consumed the same amount of skimmed milk (S; n=76) for 12 months (See Table 1 for compositions). The volunteers were recruited from different companies in the Granada metropolitan area by the researcher group of the Department of Nutrition and Health at Puleva Biotech SA, Granada, Spain. They were included after consulting their medical history (to rule out reasons for exclusion), performing a physical examination, and assessing their cardiovascular risk by interview (performed by their company medical services and taking into account age, sex, use of oral contraceptives, family history of CVD, blood pressure, body mass index, tobacco consumption, physical activity, fasting blood glucose, and total cholesterol concentration). Subjects presumably at moderate risk of developing CVD were included in the study. The exclusion criteria were: the use (at least 1 month before the study) of any medication known to influence lipid metabolism, pregnancy, any chronic or metabolic disease (including any kind of dyslipidemia), type 2 diabetes, any renal, hepatic or gastrointestinal disease, lactose intolerance, and cow's milk protein intolerance [24].

At baseline and 12 months, anthropometric variables (height, weight, body mass index) and blood pressure were recorded for all subjects. In addition, plasma glucose, triacylglycerol, total cholesterol

(TC), and high density lipoprotein (HDL) cholesterol were measured (in triplicate) by colorimetry using commercial reagents obtained from Biosystems (Barcelona, Spain). Plasma LDL cholesterol was calculated according to the formula of Friedewald et al. [25]. Total cholesterol/HDL cholesterol (TC/HDL) and LDL/HDL cholesterol ratios were used as biomarkers of CVD risk since large epidemiological and clinical studies have found them reliable predictors as well as the most appropriate variables for monitoring the effectiveness of lipid-lowering therapies [26, 27, 28, 29].

DNA extraction and genotyping

A retrospective genetic analysis of the subjects was performed. Genomic DNA from each subject was isolated from 300 µl of total blood using the QIAamp DNA Blood Mini Kit (QIAGEN, Spain). DNA was recovered in 100 µl of nuclease-free water and its concentration and quality measured using a nanodrop ND-2000 spectrophotometer. The mean concentration of the samples was 80-90 ng/µl, and the 260/280 and 260/230 absorbance ratios over 1.7, meeting the requirements for analysis using the Taqman OpenArray Genotyping Platform genotyping system and the 7900HT Fast Real-Time PCR System. TaqMan Genotyper Software v1.0.1 was used to determine the quality of genotyping; those genotypes with qualities under 90% were discarded.

Polymorphism in genes coding for proteins involved in lipid metabolism and related pathways were selected adhering to three criteria: a putative functional effect, gene coverage within tagged SNPs (Hapmap International Project, www.hapmap.org), and an allele frequency of close to 50%. Tagger web server [30] was used for SNP selection. Fourteen polymorphisms (APOB: rs104203, rs693, PLA2G4B: rs1197669, SREBF1:rs9902941, SREBF2: rs2267443, rs2229442, IL4: rs2243250, NFKBIA: rs8904, PPARA: rs135551, rs135549, rs6008259, SELE: rs5368, SELP: rs6131, rs2205895) were examined in all 161 participants.

The study was conducted according to the standards of the Helsinki Declaration. The intervention protocol was approved by the Ethics Committee of *Fundación Hospital Virgen de las Nieves* (Granada, Spain). Informed written consent to participate was obtained from all subjects.

Statistical analysis

Deviations from Hardy–Weinberg equilibrium of genotype frequencies at individual loci were assessed using standard Chi-squared tests. Significance was set at $p>0.05$. Continuous descriptive variables were expressed as means and 95% confidence intervals (95%CI). Genotype and allele frequencies were expressed as percentages.

Differences between the SS and S groups in terms of changes in the measured variables between baseline and 12 months were examined by two-way repeated-measures ANOVA. Differences in responses according to genotype were calculated by the change in TC/HDL and LDL/HDL cholesterol ratios between baseline and 12 months. Three-way repeated-measures ANOVA was used to examine the effect of time, treatment and genotype on the measured variables, followed by Bonferroni correction. Since 14 polymorphisms and two types of milk were studied, significance was set at $p<0.0017$. Genetic analyses were performed using the dominant model, in which carriers of 1 or 2 copies of the minor allele are grouped and compared with major allele homozygotes. All calculations were performed using R Statistical Software v.2.15.

RESULTS

At the end of the study, no significant differences were seen between the SS and S groups in terms of the change in any biochemical variable or CVD risk biomarker (Table 2). However, the TC/HDL and LDL/HDL ratios did show a trend towards reduction in the S group and towards an increase in the SS group.

No significant differences were seen between the S and SS groups or genotype of any studied polymorphism in terms of baseline age, BMI, TC, HDL, LDL, TC/HDL and LDL/HDL ratios, glucose, triacylglycerol, or blood pressure.

To assess inter-individual genetic variation in terms of the response to the consumption of the different types of milk, all participants were genotyped for the genetic variants shown in Table 3. All SNP genotype frequencies were in Hardy–Weinberg equilibrium ($p>0.05$).

Five polymorphisms were initially found associated with changes in the TC/HDL and LDL/HDL ratios regarding the different kind of milk consumed: PPARA rs135549; PPARA rs135551; SELE

rs5368; SREBF2 rs2229442; SELP rs6131) (Tables 4 and 5). After Bonferroni correction for multiple testing, the association involving PPARA rs135549 remained. The TT genotype of this SNP was significantly associated with a reduction in both the TC/HDL and LDL/HDL ratios after 12 months of S milk intake (Fig. 1). It was also associated (but not significantly) with reductions in LDL cholesterol and TC after S milk consumption [-6.99 (-14.75 - 0.76) and -6.06 (-14.74 - 2.63) mg/dl], while these values increased in subjects with this genotype in the SS milk group [12.25 (0.52 - 23.97) and 8.92 (-1.66 - 19.50) mg/dl] respectively.

No differences were observed in the TC/HDL and LDL/HDL ratios of C allele carriers after consuming either S or SS milk.

Neither the type of milk consumed nor genotype had any significant effect on BMI, TC, HDL, glucose, triacylglycerol or blood pressure.

DISCUSSION

The present work examines the effects of skimmed and semi-skimmed milk intake on biomarkers of risk for CVD in subjects retrospectively genotyped for polymorphisms in key genes involved in fatty acid metabolism.

The type of milk consumed (SS or S) did not significantly affect the atherogenic lipid profile of the subjects as a whole, as reported by other authors [31, 32]. However, the consumption of the S milk did have different effects depending on subject genotype (Fig. 1).

The important contributions of milk towards the coverage of human dietary requirements for energy, high quality protein and a number of key minerals and vitamins are well documented. The nutritional importance of dairy fat, however, is less well understood. There is a general perception that foods containing saturated fat are unlikely to be beneficial to health [12], but the relationship between dairy foods, particularly milk, and the risk of CVD remains unclear [11, 33]. A recent dose-response meta-analysis of 17 prospective studies, examining the relationship between total dairy intake, milk intake, low-fat and high-fat dairy intake, and the risk of CVD and all-cause mortality, indicated milk intake to be modestly and inversely associated with CVD risk [34]. The literature, however, contains few interventional studies, and those that have been performed have focused only on the effects of

different dairy products (butter, cheese, yoghurt, milk, etc.) on changes in plasma lipid concentrations. The data from these suggest different dairy products to have different effects on lipoproteins, and it is unclear what the impact of consuming reasonable amounts of dairy items on cardiovascular risk actually is [35].

The absence of agreement regarding the effect of milk consumption on CVD risk, along with negative feelings regarding the amount of saturated fatty acids in milk, have led to an overall reduction in milk consumption [17] accompanied by the systematic choice of low fat milk. However, this could have an impact on total calorie intake, especially in elderly people or the malnourished, negatively affecting their nutritional status. Further, the fat in milk is important in its palatability; many people may therefore enjoy skimmed milk much less.

In the present study, no significant differences in the measured variables were seen between the SS and S groups after 12 months, suggesting that neither type of milk has any effect on CVD risk biomarkers. It may be that the differences in the composition of the milks (just 5.64 g of saturated fat and 212.5 kJ/day) employed were insufficient for any differences in response to their consumption to become noticeable. However, the aetiology of cardiovascular disease (which is complex and multifactorial), and the efficacy of any treatment, requires a genetic exploration be made to determine effects at the individual rather than populational level [22, 23]. There is growing evidence that while universal nutritional recommendations might be appropriate for the general population, variability among persons (related to a combination of environmental and genetic factors) may render them less suitable at the individual level. The interaction between genetic and dietary components has helped in understanding this variability [36]. With further study into the interaction between the most important genetic markers or single-nucleotide polymorphisms (SNPs) and diet, it may be possible to understand variability in lipid metabolism. This could lead to the increased use of personalized nutrition recommendations to combat metabolic disorders [36].

Many studies have shown that the heterogeneity in plasma lipoprotein and lipid responsiveness to changes in dietary fat intake can be partly explained by variation in genes that code for enzymes or proteins related to lipoprotein metabolism [37, 38]. Peroxisome proliferator-activated receptors (PPARs) have emerged as one of the central regulators of gene/diet interactions with respect to fat

intake. PPARA is a ligand-dependent transcription factor that plays a key role in lipid homeostasis. Indeed, the activation of PPARA contributes to the clearance of triglyceride-rich lipoproteins, improves HDL cholesterol concentrations, and reduces the oxidation of LDL cholesterol, influencing the activity of key players in lipid metabolism such as lipoprotein lipase, apoC-III and the induction of enzymes related to fatty acid oxidation [39]. Specific genetic variants of this gene have been shown to influence lipid concentrations during fasting as well as the acute postprandial response to dietary fat [36]. In the present study, PPARA rs135549 modulated the magnitude of the effects of the S and SS milk on CVD risk biomarkers. Individuals homozygous for the major T-allele showed a reduction in the TC/HDL and LDL/HDL ratios after consuming the S milk, and an increase after consuming the SS milk ($p=0.0015$ and 0.0005 respectively) compared to carriers of the C allele. Thus, T allele homozygotes seem to benefit from S milk intake but are at a disadvantage after consuming SS milk in comparison with C allele carriers or indeed the population in general (Fig. 1).

One of the most commonly studied variants of PPARA is Leu162Val, the minor allele of which has been associated with higher fasting total cholesterol, LDL and apolipoprotein B (apoB) concentrations after a single fat load [40, 41, 42]. This suggests that the PPARA variants Leu162Val and rs135549 may both modulate the risk of CVD by influencing lipid concentrations. However, they must do this via different mechanisms since rs135549 is located in an intron while the Leu162Val variant is a missense.

Before Bonferroni correction, other polymorphisms were shown to be associated with changes in the TC/HDL and LDL/HDL ratios and the type of milk consumed: PPARA rs135551; SELE rs5368; SREBF2 rs2229442; SELP rs6131. After Bonferroni correction, however, only PPARA rs135549 remained significantly associated with the response of both ratios. The lack of a significant effect on the part of the other SNPs might be related to the fact that this was a simultaneous analysis of different SNPs in a relatively small population. Additional studies with larger sample sizes or focused on individual SNPs should be performed to clarify the role of each.

Although heterogeneity in serum lipid responsiveness to changes in dietary fat had been previously associated with genetic variation, Estévez-González et al. were the first to examine the change in serum lipids after the consumption of different types of milk, and the first to investigate the

modulation of plasma lipid concentrations associated with Taq 1B polymorphism in the cholesteryl ester transfer protein gene (CEPT-Gene). The authors identified increases in HDL cholesterol (reducing the LDL/HDL ratio) to be significantly greater in those homozygous for the major allele (B1B1) of this gene [43].

The present work advances our understanding of the role of polymorphisms in genes that modulate the responses of lipoprotein and lipid according to the type of milk consumed; the genetic results partially explain the variation in individual response seen. However, it should be remembered that milk consumption in this study was higher than the reported average consumption for the Spanish population (500mL/day vs. 1 serving/day (250ml/day) [44]. Further, the results need to be validated with studies involving larger numbers of participants. Moreover, the proportion of men was considerably greater than that of women (an effect of the recruitment procedure). Gender has been described as a determining factor in CVD risk [45]; it would therefore be of great interest to explore differences between males and females in future studies.

PPARA plays a key role in lipid homeostasis, contributing towards the regulation of lipid concentrations at different transcriptional and post-translational levels. However, the biological mechanisms underlying these effects are not well understood. Additional work is needed to analyse the specific molecular mechanisms involved in the differential regulation mediated by intronic rs135549 variants on lipid concentrations after milk consumption.

This study provides the first evidence that polymorphism in the PPARA gene (rs135549) modulates the effects of different types of milk on CVD risk biomarkers. Subjects with the TT variant (47% of the studied population) may significantly benefit from skimmed milk consumption. In subjects with the CC or C/T variants (53% of the population), however, the consumption of semi-skimmed milk or skimmed would have similar effects on TC/HDL and LDL/HDL ratios.

CONCLUSION

To our knowledge, the present study is the first to examine rs135549 polymorphism in the context of gene/diet interaction. PPARA rs135549 modulates the magnitude of the effects of skimmed and semi-skimmed milk on a range of CVD risk biomarkers. The TT genotype of rs135549 was associated with

a reduction in TC/HDL and LDL/HDL ratios after consuming skimmed milk, and an increase after consuming semi-skimmed milk. This might allow personalized nutritional recommendations to be given that could help prevent or mitigate CDV but avoid unnecessary restrictions in subjects who would not benefit from them. Though further studies with a prospective design are needed to confirm the present findings, these genotype differences might help to explain the variability of results seen in studies evaluating the effect of milk fat on CVD risk.

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REFERENCES

- [1] Smith SC Jr. Screening for high-risk cardiovascular disease: a challenge for the guidelines: comment on "systematic review of guidelines on cardiovascular risk assessment: which recommendations should clinicians follow for a cardiovascular health check?" *Arch Intern Med.* 2010, 170, 40-42.
- [2] Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb.* 1992, 12, 911-919.
- [3] Gordon T. The diet-heart idea: outline of a history. *Am J Epidemiol.* 1988, 127, 220-225.
- [4] WHO Study Group. Diet, nutrition and prevention of chronic diseases. Technical report series no. 916, World Health Organization, Geneva 2003.
- [5] Lichtenstein AH, Appel LJ, Brands M, Carnethon M, Daniels S, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation.* 2006, 114, 82-96.
- [6] Graham, I., Atar, D., Borch-Johnsen, K., Boysen, G., Burell, G. et al., European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). *Eur Heart J.* 2007, 28, 2375-414.
- [7] Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA. et al. Dietary saturated fats and their food sources in relation to the risk of coronary heart disease in women. *Am J Clin Nutr.* 1999, 70, 1001-1008.
- [8] Patterson E, Larsson SC, Wolk A, Akesson A. Association between Dairy Food Consumption and Risk of Myocardial Infarction in Women Differs by Type of Dairy Food. *J Nutr.* 2013, 14, 74-9.
- [9] Kliem KE, Givens DI. Dairy products in the food chain: their impact on health. *Annu Rev Food Sci Technol.* 2011, 2, 21-36.

- 317 [10] Larsson SC., Virtamo J, Wolk A. Dairy consumption and risk of stroke in Swedish women and
318 men. *Stroke*. 2012, 43, 1775-80.
- 319 [11] Lamarche B. Review of the effect of dairy products on non-lipid risk factors for cardiovascular
320 disease. *J Am Coll Nutr*. 2008, 27, 741S-6S.
- 321 [12] German JB, Gibson RA, Krauss RM, Nestel P, Lamarche B. et al. A reappraisal of the impact of
322 dairy foods and milk fat on cardiovascular disease risk. *Eur J Nutr*. 2009, 48, 191-203
- 323 [13] Huth PJ, Park KM. Influence of dairy product and milk fat consumption on cardiovascular
324 disease risk: a review of the evidence. *Adv Nutr*. 2012, 3,266-285.
- 325 [14] Soedamah-Muthu, S. S., Masset, G., Verberne, L., Geleijnse, J. M., Brunner, E. J., Consumption
326 of dairy products and associations with incident diabetes, CHD and mortality in the Whitehall II study.
327 *Br J Nutr*. 2012, 7, 1-9.
- 328 [15] Elwood PC, Pickering JE, Givens DI, Gallacher JE. The consumption of milk and dairy foods and
329 the incidence of vascular disease and diabetes: an overview of the evidence. *Lipids*. 2010, 45, 925-
330 939.
- 331 [16] Corella D, Arregui M, Coltell O, Portolés O, Guillem-Sáiz P. et al. Association of the LCT-
332 13910C>T polymorphism with obesity and its modulation by dairy products in a Mediterranean
333 population. *Obesity (Silver Spring)*. 2011, 19, 1707-14.
- 334 [17] van Staveren WA, Steijns JM, de Groot LC. Dairy products as essential contributors of (micro-)
335 nutrients in reference food patterns: an outline for elderly people. *J Am Coll Nutr*. 2008, 27, 747S-
336 54S.
- 337 [18] German JB, Dillard CJ. Composition, structure and absorption of milk lipids: a source of energy,
338 fat-soluble nutrients and bioactive molecules. *Crit Rev Food Sci Nutr*. 2006,46, 57-92.
- 339 [19] Contarini G, Povolo M. Phospholipids in milk fat: composition, biological and technological
340 significance, and analytical strategies. *Int J Mol Sci*. 2013, 14, 2808-31.

- 341 [20] Corella D, Ordovas JM. Nutrigenomics in cardiovascular medicine. *Circ Cardiovasc Genet*. 2009,
342 2, 637-51.
- 343 [21] Simopoulos AP. Nutrigenetics/nutrigenomics. *Annu Rev Public Health*. 2010, 31, 53–68.
- 344 [22] Bouchard C, Ordovas JM. Fundamentals of nutrigenetics and nutrigenomics. *Prog Mol Biol*
345 *Transl Sci*. 2012, 108, 1-15.
- 346 [23] Zeisel SH, Waterland RA, Ordovás JM, Muoio DM, Jia W, et al. Highlights of the 2012 Research
347 Workshop: Using nutrigenomics and metabolomics in clinical nutrition research. *JPEN J Parenter*
348 *Enteral Nutr*. 2013, 37,190-200.
- 349 [24] Fonollá J, López-Huertas E, Machado FJ, Molina D. Alvarez, I. et al., Milk enriched with
350 "healthy fatty acids" improves cardiovascular risk markers and nutritional status in human volunteers.
351 *Nutrition*. 2009, 25, 408-14.
- 352 [25] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density
353 lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 1972, 18,
354 499–502.
- 355 [26] Manninen V, Tenkanen L, Koskinen P, Huttunen JK, Manttari M, et al. Joint effects of serum
356 triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in
357 the Helsinki Heart Study. Implications for treatment. *Circulation*. 1992, 85, 37– 45.
- 358 [27] Cullen P, Schulte H, Assmann G. The Munster Heart Study (PROCAM) Total Mortality in
359 Middle-Aged Men is increased at low total and LDL cholesterol concentrations in smokers but not in
360 nonsmokers. *Circulation* 1997, 6, 2128– 2136.
- 361 [28] Kannel WB. Risk stratification of dyslipidemia: Insights from the Framingham Study. *Curr Med*
362 *Chem Cardiovasc Hematol Agents*. 2005, 3, 187– 193.

- 363 [29] Millán J, Pintó X, Muñoz A, Zúñiga M, Rubiés-Prat J, et al. Lipoprotein ratios: Physiological
364 significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag.* 2009, 5,
365 757-65.
- 366 [30] de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly M.J, et al. Efficiency and power in genetic
367 association studies. *Nat. Genet.* 2005, 37, 1217-1223.
- 368 [31] Tholstrup T, Sandström B, Hermansen JE, Hølmer G. Effect of modified dairy fat on postprandial
369 and fasting plasma lipids and lipoproteins in healthy young men. *Lipids.* 1998, 33, 11-21.
- 370 [32] Jacques H, Gascon A, Arul J, Boudreau A, Lavigne C, et al. Modified milk fat reduces plasma
371 triacylglycerol concentrations in normolipidemic men compared with regular milk fat and
372 nonhydrogenated margarine. *Am J Clin Nutr* 1999, 70, 983-991.
- 373 [33] Soedamah-Muthu SS, Ding EL, Al-Delaimy WK, Hu FB, Engberink MF, et al. Milk and dairy
374 consumption and incidence of cardiovascular diseases and all-cause mortality: dose-response meta-
375 analysis of prospective cohort studies. *Am J Clin Nutr.* 2011, 93, 158-71.
- 376 [34] van Aarde MA, Soedamah-Muthu SS, Geleijnse JM, Snijder MB, Nijpels G, et al. Dairy intake in
377 relation to cardiovascular disease mortality and all-cause mortality: the Hoorn Study. *Eur J Nutr.* 2013,
378 52, 609-16.
- 379 [35] Garcia-Rios A, Pérez-Martínez P, Delgado-Lista J, Lopez-Miranda J, Perez-Jimenez F.
380 Nutrigenetics of the lipoprotein metabolism. *Mol Nutr Food Res.* 2012, 56, 171-83.
- 381 [36] Ordovas JM, Lopez-Miranda J, Mata P, Perez-Jimenez F, Lichtenstein AH, et al. Gene-diet
382 interaction in determining plasma lipid response to dietary intervention. *Atherosclerosis* 1995, 118,
383 S11-27.
- 384 [37] Ohlsson L. Dairy products and plasma cholesterol levels. *Food Nutr Res.* 2010, 54. doi:
385 10.3402/fnr.v54i0.5124

- [38] Ordovas JM. The genetics of serum lipid responsiveness to dietary interventions. *Proc Nutr Soc* 1999,58, 171– 87.
- [39] Tai ES, Demissie S, Cupples LA, Corella D, Wilson PW, et al. Association between the PPARA L162 V polymorphism and plasma lipid levels: the Framingham Offspring Study. *Arterioscler. Thromb Vasc Biol.* 2002, 22, 805–810.
- [40] Hamblin M, Chang L, Fan Y, Zhang J, Chen YE. PPARs and the cardiovascular system. *Antioxid. Redox. Signal.* 2009, 11, 1415–1452.
- [41] Paradis AM, Fontaine-Bisson B, Bossé Y, Robitaille J, Lemieux S, et al. The peroxisome proliferator-activated receptor alpha Leu162Val polymorphism influences the metabolic response to a dietary intervention altering fatty acid proportions in healthy men. *Am J Clin Nutr.* 2005, 81, 523-530.
- [42] Tanaka T, Ordovas JM, Delgado-Lista J, Perez-Jimenez F, Marin C, et al. Peroxisome proliferator-activated receptor alpha polymorphisms and postprandial lipemia in healthy men. *J Lipid Res.* 2007, 48, 1402–1408.
- [43] Estévez-González MD, Saavedra-Santana P, López-Ríos L., Chirino R., Cebrero-García E, et al. HDL cholesterol levels in children with mild hypercholesterolemia: effect of consuming skim milk enriched with olive oil and modulation by the TAQ 1B polymorphism in the CETP gene. *Ann Nutr Metab.* 2010, 56, 288-93.
- [44] Varela-Moreiras G, Avila JM, Cuadrado C, del Pozo S, Ruiz E, Moreiras O. Evaluation of food consumption and dietary patterns in Spain by the Food Consumption Survey: updated information. *Eur J Clin Nutr.* 2010, 64, S37-43.
- [45] Mosca L., Benjamin EJ, Berra K., Bezanson JL, Dolor RJ, et al. Effectiveness-based guidelines for the prevention of cardiovascular disease in women--2011 update: a guideline from the American Heart Association. *Circulation.* 2011, 123, 1243-62.

411 **Figure 1:** Comparison of the change in the TC/HDL (A) and LDL/HDL (B) ratios between baseline
412 and 12 months for the population as a whole ($p>0.05$), and by PPARA rs135549 genotype, according
413 to the type of milk consumed. Subjects with the TT genotype showed reduced TC/HDL and
414 LDL/HDL ratios after S milk consumption, and increased ratios after SS milk consumption ($p=0.0015$
415 and 0.0005 respectively). No differences were observed in C carriers ($p>0.051$). Data are means at
416 baseline (t0) and 12 months (t12).

Table 1. Composition of semi-skimmed and skimmed milks.

	Semi-skimmed milk (SS) ^a	Skimmed milk (S) ^a
Energy (Kcal)	232.50	175.00
Fat (g)	9.50	1.50
SFAs (g)	6.69	1.05
MUFAs (g)	2.58	0.40
PUFAs (g)	0.21	0.03
Protein (g)	15.50	16.00
Carbohydrates (g)	23.50	24.00

^a The composition matches the volume of milk consumed daily during the intervention study: (500 mL/d). SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids.

Table 2. Change in biochemical variables and cardiovascular risk biomarkers after SS and S milk consumption for 12 months ^a

	Group	T ₀	T ₁₂	p-time	P-milk	P-time*milk
BMI	SS	28.08 (27.14 - 29.02)	28.47 (27.49 - 29.46)	0.01	0.38	0.59
(kg/m ²)	S	28.75 (27.77 - 29.73)	29 (28.06 - 29.94)			
TC	SS	218.54 (211.17 - 225.91)	223.68 (216.3 - 231.07)	0.26	0.22	0.17
(mg/dl)	S	215.56 (208.24 - 222.88)	214.83 (207.67 - 221.98)			
HDL	SS	44.5 (41.92 - 47.08)	44.45 (41.55 - 47.34)	0.87	0.78	0.22
(mg/dl)	S	44.44 (41.88 - 46.99)	45.25 (42.63 - 47.87)			
LDL	SS	143.27 (135.46 - 151.08)	147.59 (138.7 - 156.49)	0.56	0.60	0.1
(mg/dl)	S	143.71 (135.64 - 151.79)	141.51 (134.05 - 148.97)			
TC/HDL	SS	5.19 (4.9 - 5.48)	5.43 (5.06 - 5.79)	0.24	0.48	0.08
	S	5.17 (4.82 - 5.52)	5.11 (4.71 - 5.51)			
LDL/HDL	SS	3.41 (3.16 - 3.66)	3.55 (3.25 - 3.85)	0.77	0.58	0.07
	S	3.43 (3.16 - 3.71)	3.32 (3.06 - 3.57)			
TG	SS	153.84 (129.21 - 178.46)	158.22 (127.48 - 188.95)	0.87	0.20	0.8
(mg/dl)	S	137.04 (115.96 - 158.12)	141.2 (115.98 - 166.41)			
Glu	SS	98.46 (93.51 - 103.41)	101.02 (96.02 - 106.01)	0.00	0.65	0.57
(mg/dl)	S	101.43 (92.53 - 110.33)	104.96 (96.68 - 113.24)			
SBP	SS	79.03 (76.5 - 81.56)	76.21 (73.79 - 78.63)	9.00E-04	0.81	0.77
(mmHg)	S	78.93 (76.03 - 81.82)	75.58 (73.4 - 77.76)			
DBP	SS	122.04 (118.26 - 125.83)	122.13 (118.54 - 125.72)	0.15	0.28	0.13
(mmHg)	S	121.59 (117.78 - 125.41)	117.75 (114.42 - 121.09)			

^a All values are means; 95% CIs in brackets. CVR: cardiovascular risk. SBP: Systolic blood pressure. DBP: Diastolic blood pressure. T₀: baseline. T₁₂: after 12 months. Semi-skimmed milk (SS). skimmed milk (S).

Table 3. Genotypic and allelic distribution of polymorphisms.

Gene	SNP	Major allele	Heterozygote ^a	Minor allele	Allele	Allele 2 ^a	HWE ^b
Symbol		Homozygote ^a		Homozygote ^a	1 ^a		p
APOB	rs693	28.8	54	17.3	55.8	44.2	0.185
APOB	rs1042031	61	33.1	5.8	77.6	22.4	0.667
PLA2G4B	rs1197669	40.2	48	11.8	64.2	35.8	0.564
SREBF1	rs9902941	33.4	49.3	17.2	58.1	41.9	0.824
SREBF2	rs2229442	85.8	13.9	0.3	92.7	7.3	0.918
SREBF2	rs2267443	32.8	45.4	21.8	55.5	44.5	0.968
IL4	rs2243250	69.1	27.7	3.2	83	17	0.631
NFKBIA	rs8904	30.4	47.3	22.3	54.1	45.9	0.352
PPARA	rs6008259	71.1	25.5	3.4	83.9	16.1	0.371
PPARA	rs135551	48.6	42.8	8.6	70	30	0.751
PPARA	rs135549	47.3	34.2	18.5	64.4	35.6	0.575
SELE	rs5368	83	16.1	0.9	91	9	0.899
SELP	rs6131	65	28.7	6.2	79.4	20.6	0.178
SELP	rs2205895	48	41.4	10.6	68.7	31.3	0.408

SNP: single-nucleotide polymorphisms. HWE: Hardy-Weinberg equilibrium. ^aPercentage ^b X² test for HWE .

Table 4. Change in the TC/HDL ratio with respect to milk consumption group and genotype.

			Change in TC/HDL				p-value	Post-Hoc	
		Groups	n	0	n	1+2	0	1+2	
APOB	rs1042031	SS	53	0.3 (0.02 - 0.57)	29	0.03 (-0.34 - 0.41)	0.7982		
		S	42	0.01 (-0.22 - 0.24)	30	-0.16 (-0.7 - 0.38)			
APOB	rs693	SS	18	-0.23 (-0.61 - 0.16)	64	0.32 (0.06 - 0.58)	0.1317		
		S	23	-0.03 (-0.68 - 0.61)	52	-0.06 (-0.29 - 0.17)			
PLA2G4B	rs1197669	SS	28	0.09 (-0.3 - 0.48)	57	0.31 (0.04 - 0.58)	0.3033		
		S	29	0.04 (-0.45 - 0.54)	45	-0.11 (-0.38 - 0.17)			
SREBF1	rs9902941	SS	25	-0.16 (-0.52 - 0.2)	58	0.41 (0.14 - 0.68)	0.9828		
		S	25	-0.42 (-0.73 - -0.11)	49	0.14 (-0.2 - 0.48)			
SREBF2	rs2267443	SS	27	0.15 (-0.19 - 0.49)	58	0.28 (-0.01 - 0.56)	0.5444		
		S	26	0 (-0.57 - 0.56)	50	-0.09 (-0.33 - 0.15)			
SREBF2	rs2229442	SS	76	0.23 (-0.01 - 0.47)	8	0.34 (-0.33 - 1.01)	0.0569	0.0357	0.0160
		S	59	0.09 (-0.19 - 0.37)	12	-0.8 (-1.27 - -0.34)			
IL4	rs2243250	SS	50	0.38 (0.06 - 0.71)	33	0.02 (-0.26 - 0.3)	0.2639		
		S	56	-0.07 (-0.35 - 0.21)	19	-0.01 (-0.56 - 0.54)			
NFKBIA	rs8904	SS	23	0.47 (0.02 - 0.91)	62	0.15 (-0.1 - 0.41)	0.5556		
		S	25	0 (-0.6 - 0.6)	50	-0.09 (-0.33 - 0.14)			
PPARA	rs135551	SS	40	0.62 (0.27 - 0.96)	40	-0.11 (-0.39 - 0.17)	0.0273	0.0057	0.7512
		S	35	-0.07 (-0.39 - 0.26)	40	-0.03 (-0.41 - 0.35)			
PPARA	rs135549	SS	30	0.52 (0.19 - 0.86)	55	0.09 (-0.2 - 0.37)	0.0248	0.0015*	0.9308
		S	27	-0.29 (-0.63 - 0.05)	49	0.07 (-0.27 - 0.4)			
PPARA	rs6008259	SS	59	0.15 (-0.14 - 0.43)	26	0.45 (0.15 - 0.75)	0.7137		
		S	52	-0.11 (-0.34 - 0.12)	24	0.05 (-0.56 - 0.67)			
SELP	rs6131	SS	52	0.07 (-0.17 - 0.3)	32	0.53 (0.09 - 0.97)	0.1647		
		S	43	-0.05 (-0.29 - 0.18)	33	-0.07 (-0.56 - 0.42)			
SELP	rs2205895	SS	41	0.57 (0.23 - 0.92)	44	-0.07 (-0.33 - 0.18)	0.0728		
		S	38	-0.04 (-0.43 - 0.35)	38	-0.08 (-0.4 - 0.23)			
SELE	rs5368	SS	71	0.3 (0.05 - 0.54)	14	-0.07 (-0.55 - 0.41)	0.0222	0.00867	0.3292
		S	63	-0.13 (-0.33 - 0.06)	12	0.54 (-0.55 - 1.63)			

All values are means; 95% CIs in brackets. Dominant model 0 = Major allele Homozygote 1+2= Heterozygote + Minor allele Homozygote.

Semi-skimmed milk (SS). skimmed milk (S). * P<0.0017

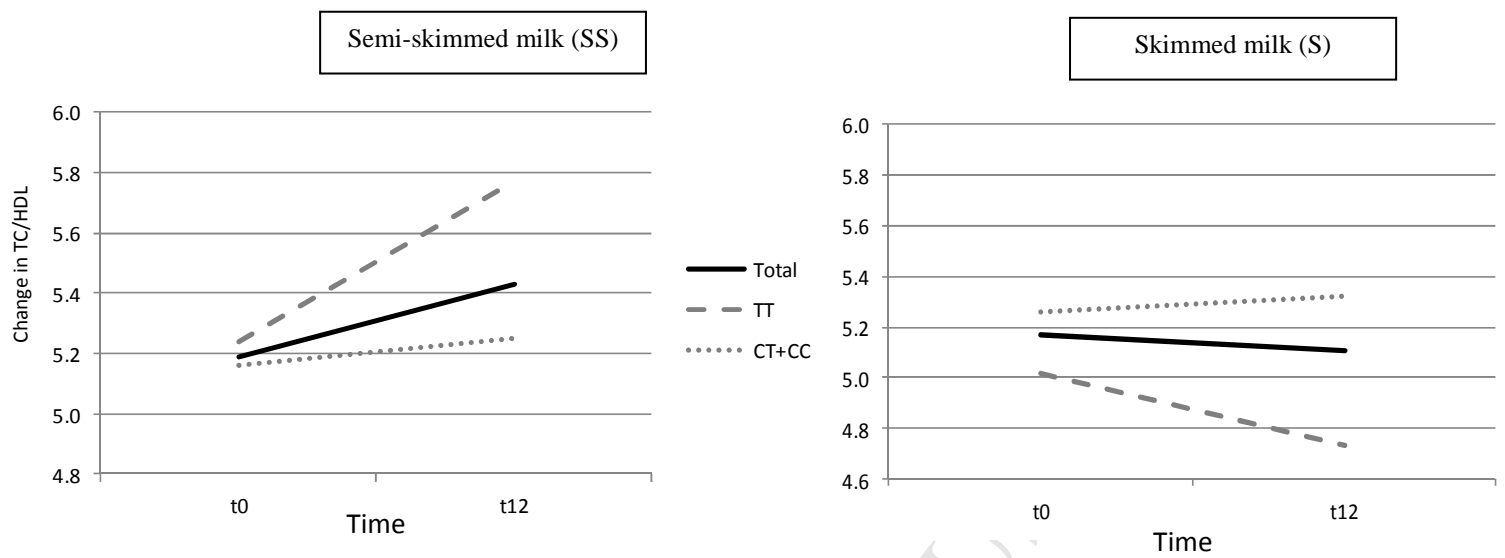
Table 5: Change in the LDL/HDL ratio with respect to milk consumption group and genotype.

				Change in_LDL/HDL			p-value	Post-Hoc	
		Group	n	0	n	1+2		0	1+2
APOB	rs1042031	SS	53	0.25 (0 - 0.5)	29	-0.21 (-0.55 - 0.12)	0.2243		
		S	42	-0.08 (-0.26 - 0.11)	30	-0.19 (-0.53 - 0.16)			
APOB	rs693	SS	18	-0.15 (-0.55 - 0.25)	64	0.19 (-0.06 - 0.43)	0.3032		
		S	23	-0.11 (-0.5 - 0.27)	52	-0.11 (-0.3 - 0.08)			
PLA2G4B	rs1197669	SS	28	0.11 (-0.25 - 0.47)	57	0.15 (-0.11 - 0.41)	0.7491		
		S	29	-0.07 (-0.32 - 0.17)	45	-0.13 (-0.37 - 0.11)			
SREBF1	rs9902941	SS	25	-0.03 (-0.41 - 0.36)	58	0.21 (-0.05 - 0.46)	0.9193		
		S	25	-0.29 (-0.59 - 0.02)	49	-0.02 (-0.23 - 0.19)			
SREBF2	rs2267443	SS	27	0.1 (-0.23 - 0.42)	58	0.16 (-0.11 - 0.42)	0.921		
		S	26	-0.18 (-0.46 - 0.1)	50	-0.08 (-0.3 - 0.13)			
SREBF2	rs2229442	SS	76	0.12 (-0.1 - 0.35)	8	0.33 (-0.27 - 0.93)	0.0266	0.4600	0.0140
		S	59	0.02 (-0.16 - 0.19)	12	-0.75 (-1.21 - -0.28)			
IL4	rs2243250	SS	50	0.22 (-0.09 - 0.54)	33	0 (-0.25 - 0.25)	0.4076		
		S	56	-0.12 (-0.3 - 0.05)	19	-0.09 (-0.55 - 0.38)			
NFKBIA	rs8904	SS	23	0.19 (-0.29 - 0.67)	62	0.12 (-0.11 - 0.35)	0.6822		
		S	25	-0.16 (-0.49 - 0.18)	50	-0.1 (-0.3 - 0.1)			
PPARA	rs135551	SS	40	0.39 (0.04 - 0.73)	40	-0.1 (-0.36 - 0.16)	0.1129		
		S	35	-0.09 (-0.37 - 0.19)	40	-0.12 (-0.33 - 0.09)			
PPARA	rs135549	SS	30	0.47 (0.16 - 0.79)	55	-0.04 (-0.3 - 0.23)	0.006	0.0005*	0.8844
		S	27	-0.31 (-0.58 - -0.03)	49	-0.01 (-0.23 - 0.2)			
PPARA	rs6008259	SS	59	0.01 (-0.25 - 0.28)	26	0.44 (0.13 - 0.74)	0.1438		
		S	52	-0.11 (-0.32 - 0.1)	24	-0.13 (-0.43 - 0.18)			
SELP	rs6131	SS	52	-0.02 (-0.24 - 0.21)	32	0.41 (0 - 0.82)	0.0442	0.8299	0.0220
		S	43	-0.05 (-0.24 - 0.14)	33	-0.2 (-0.5 - 0.1)			
SELP	rs2205895	SS	41	0.34 (0.01 - 0.66)	44	-0.05 (-0.3 - 0.21)	0.1572		
		S	38	-0.12 (-0.34 - 0.09)	38	-0.11 (-0.38 - 0.16)			
SELE	rs5368	SS	71	0.19 (-0.04 - 0.43)	14	-0.15 (-0.59 - 0.29)	0.0864	0.0030	0.0300
		S	63	-0.13 (-0.3 - 0.04)	12	0.17 (-0.25 - 0.6)			

All values are means; 95% CIs in brackets. Dominant model 0 = Major allele Homozygote 1+2 = Heterozygote + Minor allele Homozygote.

Semi-skimmed milk (SS). skimmed milk (S). * P<0.0017

A)



B)

