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Long-chain n-3 PUFA supplied by the usual diet decrease plasma stearoyl-CoA desaturase index in non-hypertriglyceridemic older adults at high vascular risk.

Ana M Pérez-Heras[1,2,*], Jordi Mayneris-Perxachs[3,*], Montserrat Cofán[1,2], Mercè Serra-Mir[1,2], Ana I. Castellote[2,3], Carmen López-Sabater[2,3], Montserrat Fitó[2,4], Jordi Salas-Salvadó[2,5], Miguel-Ángel Martínez-González[2,6], Dolores Corella[2,7], Ramon Estruch[2,8], Emilio Ros[1,2] and Aleix Sala-Vila[1,2]

From [1] Lipid Clinic, Endocrinology and Nutrition Service, Biomedical Research Institute August Pi i Sunyer (IDIBAPS), Hospital Clínic, Barcelona; [2] CIBER Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), Madrid; [3] Dept. of Nutrition and Food Science, Reference Center in Food Technology, Faculty of Pharmacy, University of Barcelona, Barcelona; [4] Cardiovascular and Nutrition Research Group, Institut de Recerca Hospital del Mar, Barcelona; [5] Human Nutrition Unit, Hospital Universitari Sant Joan de Reus, IISPV, Universitat Rovira i Virgili, Reus; [6] Department of Preventive Medicine and Public Health, School of Medicine, University of Navarra–Clínica Universidad de Navarra, Pamplona; [7] Department of Preventive Medicine and Public Health, School of Medicine, University of Valencia, Valencia; [8] Department of Internal Medicine, IDIBAPS, Hospital Clinic, Barcelona - all in Spain.

* These authors contributed to this work equally.

Corresponding author: Aleix Sala-Vila, PhD, Clínica de Lípids, Hospital Clínic, Villarroel 170, Edifici Helios, despatx 8, 08036 Barcelona, Spain. Tel. +34932275400 ext. 2276. Fax. +34934537829 E-mail: asala@clinic.ub.es

ABSTRACT

Background and aims: The activity of stearoyl-CoA desaturase-1 (SCD1), the central enzyme in the synthesis of monounsaturated fatty acids (MUFA), has been associated with *de novo* lipogenesis. In experimental models SCD1 is down-regulated by polyunsaturated fatty acids (PUFA), but clinical studies are scarce. The effect of long-chain n-3 PUFA (LCn-3PUFA) supplied by the regular diet, in the absence of fatty fish or fish oil supplementation, remains to be explored.

Methods: We related 1-y changes in plasma SCD1 index, as assessed by the C16:1n-7/C16:0 ratio, to both adiposity traits and nutrient intake changes in a sub-cohort (n = 243) of non-hypertriglyceridemic subjects of the PREDIMED (PREvención con DIeta MEDiterranea) trial.

Results: After adjustment for confounders, including changes in fasting triglycerides, plasma SCD1 index increased in parallel with body weight (0.221 [95% confidence interval, 0.021 to 0.422], P = 0.031) and BMI (0.115 [0.027 to 0.202], P = 0.011). Additionally, dietary LCn-3PUFA (but not MUFA or plant-derived PUFA) were associated with decreased plasma SCD1 index (–0.544 [–1.044 to –0.043], P = 0.033, for each 1 g/d-increase in LCn-3PUFA). No associations were found for other food groups, but there was a trend for fatty fish intake (–0.083 [–0.177 to 0.012], P = 0.085, for each 10 g/d-increase).

Conclusions: Our data add clinical evidence on the down-regulation of plasma SCD1 index by LCn-3PUFA in the context of realistic changes in fish consumption in the customary, non-supplemented diet.

Clinical Trial Registration: http://www.Controlled-trials.com/ISRCTN35739639

INTRODUCTION

De novo lipogenesis (DNL) allows the organism to safely store the surplus of carbohydrate-supplied energy [1]. In this process, which involves a cross-talk between liver and adipose tissue, the microsomal enzyme stearoyl-CoA desaturase-1 (SCD1) plays a key role in synthesizing monounsaturated fatty acids (MUFA) (palmitoleic [C16:1n-7] and oleic [C18:1n-9]) from their saturated precursors [2].

Organ-specific SCD1 activity can be measured directly in liver biopsies or indirectly using stable isotopes [3]. Otherwise, enzyme activity can be estimated by the so-called SCD1 index, based on the ratios C16:1n-7/C16:0 or C18:1n-9/C18:0 in circulating lipids [3]. Epidemiologic observations of direct associations between the SCD1 index and metabolic diseases [4-6] and total and cardiovascular mortality [7] prompted the notion that SCD1 inhibition could be a promising strategy for the management of diseases related to DNL [2]. However, conflicting results were obtained in experimental models after knocking-down SCD1, in particular accelerated atherosclerosis in spite of amelioration of metabolic syndrome traits [8]. As a result, there is increasing consensus that, rather than causally contributing to metabolic disease, SCD1 might be “the lesser evil” (i.e., it prevents liver damage by disposing of excess lipotoxic saturated fatty acids [3]), SCD1 expression being a mere surrogate of DNL [9].

The SCD1 gene is highly regulated at the transcriptional level by hormones and nutrients [2], particularly cholesterol, simple carbohydrates and dietary polyunsaturated fatty acids (PUFA) [3,10]. Most evidence on SCD1 regulation has been obtained in experimental models, while clinical studies are scarce. In this regard, results from feeding intervention trials showed that consumption of a diet enriched in plant-derived PUFA decreased SCD1 index compared to a diet high in

SFA [11-13] or a low-fat diet [14]. The effect of long-chain n-3 PUFA (LCn-3PUFA, namely eicosapentaenoic [C20:5n-3, EPA] and docosahexaenoic acids [C22:6n-3, DHA]) on SCD1 index has been investigated only after pharmacological doses (2.4 g/d) [15], consumption of 150 g/d of cod or salmon [16], or advice to follow a diet rich in fatty fish (3.3 g LCn-3PUFA/1600 kcal) [17]. However, the effect on SCD1 of LCn-3PUFA supplied by the regular diet remains to be explored. We hypothesized that increased intake of LCn-3PUFA through a non-supplemented diet would be associated with lower SCD1 index. To address this issue, we explored the associations between 1-y changes in plasma C16:1n-7/C16:0 ratio with food and nutrient intake changes in a sample of older subjects at high cardiovascular risk participating in a nutrition intervention trial.

MATERIALS AND METHODS

Study population

This is a post-hoc analysis of a sub-sample of participants in the PREDIMED (PREvención con DIeta MEDiterránea) trial (http://www.predimed.es) (http://www.Controlled-trials.com/ISRCTN35739639) [18]. From October 2003 to June 2009, a total of 8713 candidates were screened for eligibility. Participants were men (55 to 80 years) and women (60 to 80 years) at high cardiovascular risk but no cardiovascular disease at enrolment. Inclusion criteria were the presence of either type 2 diabetes or at least three of the following risk factors: current smoking; hypertension; LDL-cholesterol ≥160 mg/dl; HDL-cholesterol ≤40 mg/dl in men or ≤50 mg/dl in women, independently of lipid-lowering therapy; BMI ≥25 kg/m^2; and family history of premature coronary heart disease. The study protocol was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the ethics committee of the recruitment centres. This sub-study was performed using data from participants with complete information on clinical characteristics, food and nutrient consumption, and plasma fatty acid composition at baseline and after intervention for ≈1 year (1.05 ± 0.15 years) (n = 391 participants).

Assessment of risk factors

Participants were considered as diabetic, hyperlipidemic or hypertensive if they had a previous diagnosis of these conditions and/or they were treated with antidiabetic, cholesterol-lowering, or antihypertensive agents, respectively. Smoking status was categorized into never, current or past smoking according to self-reports. Physical activity was determined with the validated Spanish version of the Minnesota Leisure-

Time Physical Activity questionnaire [19]. Trained personnel measured height using a wall-mounted stadiometer, body weight by calibrated scales, and waist circumference by using an anthropometric tape midway between the lowest rib and the iliac crest. Systolic and diastolic blood pressures were measured in triplicate with a validated semi-automatic oscillometer (Omron HEM-705CP; Hoofddorp, The Netherlands).

Dietary assessment and interventions

The dietary habits of participants were assessed using a validated 137-item food frequency questionnaire (FFQ) including eight items related to seafood [20]. The FFQ was completed by a trained dietician in face-to-face interviews and nutrient intakes were computed using Spanish food composition tables [21].

Candidates were randomly assigned to one of the three interventions: Mediterranean diet supplemented with extra-virgin olive oil, Mediterranean diet supplemented with mixed nuts, or advice to follow a low-fat (control) diet. The first two groups received intensive education to follow the Mediterranean diet and free allotments of either extra-virgin olive oil (1 L/week) or 30 g/day of mixed nuts (15 g walnuts, 7.5 g hazelnuts and 7.5 g almonds). Participants in the control group were given intensive advice on how to follow a low-fat diet and received non-food gifts. At baseline and quarterly, dieticians run individual and group sessions separately for each group. In each session, a dietary screener of adherence to the Mediterranean diet was used to assess compliance [22].

Biochemical analyses

Fasting blood samples were collected at baseline and at 1 y of follow-up. Serum lipid and glucose concentrations were determined by standard enzymatic methods in the hospital clinical laboratory. The plasma fatty acid profile was determined by gas-chromatography as described [23]. Plasma SCD1 index was estimated as the ratio C16:1n-7/C16:0. Given the selective C16:1n-7 and C16:0 partitioning in plasma triglycerides (TG), the use of C16:1n-7/C16:0 ratio in total plasma in subjects with hypertriglyceridemia (defined as a fasting TG >150 mg/dL) has been cautioned against [24]. We therefore excluded subjects with hypertriglyceridemia either at baseline or at the end of the study (n = 148). Subsequent data refer only to normotriglyceridemic completers (n = 243).

Statistical methods

When appropriate, the ANOVA or chi-square tests were used to assess whether the clinical characteristics of normotriglyceridemic completers were comparable to those of the whole samples of study participants. We constructed multivariate linear regression models to investigate independent associations between 1-y changes in weight, BMI and waist (dependent variables) and 1-y changes in plasma SCD1 index, adjusting for the baseline values of each dependent variable, age, gender, 1-year changes in fasting TG, 1-y changes in physical activity, 1-y changes in energy intake, and 1-y changes in HDL-cholesterol. Given that changes in plasma TG occur in parallel with changes in plasma SCD1 index [24], we also included in the model the interaction between the two variables. Similarly, we constructed multivariate linear regression models to search for independent determinants of 1-y changes in plasma SCD1 index (dependent variable), adjusting for age, gender, 1-year changes in fasting TG, 1-y changes in physical activity, and 1-y changes in HDL-cholesterol.

The model also included simultaneous dietary variables (1-y changes in intakes of energy, protein, total carbohydrates, simple sugars, cholesterol, SFA, MUFA, plant-derived PUFA [C18:2n-6 + C18:3n-3], LCn-3PUFA [EPA + DHA], and alcohol). To determine whether an eventual association between plasma SCD1 index and LCn-3PUFA was mediated through the TG-lowering effect of LCn-3PUFA, we constructed a second multivariate model including the interaction between 1-y changes in LCn-3PUFA and 1-y changes in TG. Alternatively, we replaced changes in nutrients by changes in consumption of vegetables, fruit, cereals, pulses, dairy products, meat and processed meat, sources of simple sugars (sodas, sweets, and pastries), wine, fatty fish, and lean fish.

Statistical significance was set at the $p<0.05$ level. Analyses were done using SPSS statistical software, version 19 (IBM Corp).

175 **RESULTS**

176 Baseline characteristics of the overall population are shown in **Table 1**. The subset
177 resulting from the exclusion of 148 participants with elevated TG had similar
178 characteristics than those of the overall sample, except for higher serum HDL-
179 cholesterol. Baseline intake and 1-year changes in intake of nutrients and selected
180 foods are shown in **Table 2. Supplementary Table 1** depicts baseline and 1-y
181 changes in plasma fatty acids. **Table 3** summarises independent determinants of 1-y
182 changes in main adiposity traits (weight, BMI and waist). We found a direct
183 association between changes in plasma SCD1 index and both changes in weight
184 and BMI. **Table 4** depicts dietary determinants of plasma SCD1 index as assessed
185 by multivariate linear regression analyses after adjusting for sex, age, 1-y changes in
186 fasting TG, cholesterol HDL, and physical activity. No significant associations were
187 found for 1-year changes in any nutrient, except for LCn-3PUFA (model 1). The
188 significance of the association lasted after inclusion of the interaction between 1-y
189 changes in LCn-3PUFA and 1-y changes in TG. The effects of 1-y changes in food
190 groups on plasma SCD1 index (model 1) are shown in **Table 5**. Changes in fatty fish
191 related to changes in plasma SCD1 index with borderline significance regardless of
192 the inclusion of the interaction between 1-y changes in fatty fish and 1-y changes in
193 fasting TG.
194

DISCUSSION

In this longitudinal 1-year study conducted in free-living non-hypertriglyceridemic individuals at high cardiovascular risk from a Mediterranean country, we found that plasma SCD1 index, a surrogate marker of DNL, increased in parallel with both changes in body weight and BMI. Additionally, dietary LCn-3PUFA (but not MUFA or plant-derived PUFA) were associated with decreased plasma SCD1 index. No effects were found for other food groups except for a borderline significant inverse association with fatty fish intake.

The link between dietary fat quality and cell membrane fatty acid composition and the extent to which it affects cellular functions and metabolic health and disease is a subject of increasing interest. In this post-hoc analysis of a sub-sample of the PREDIMED trial, we report an inverse association between changes in dietary LCn-3PUFA intake and the plasma SCD1 index. This adds to the increasing evidence of dietary LCn-3PUFA as a sound strategy in the management of obesity-associated metabolic disorders (as reviewed in [25]). Or results concur with those of clinical trials of daily supplementation with EPA+DHA (2.4 g/d) [15], 150-g cod or salmon servings [16], and the advice to include fatty fish in the diet (3.3 g LCn-3PUFA/1600 kcal) [17]. However, in relation to our findings, two points deserve to be underlined. First, the effect on plasma SCD1 index that we observed was in the context of dietary changes in LCn-3PUFA intake achieved through a normal diet. This adds to the notion of fish-derived LCn-3PUFA as a healthy fat [26]. Second, we speculate that intake of these fatty acids not only appears to down-regulate SCD1, but also might help counteract its side-effects. Of note, hyperlipidemic mice treated with antisense oligonucleotides to inhibit SCD1 activity improved metabolic syndrome traits but, surprisingly, displayed accelerated atherosclerosis [8]. This was believed

to be due to a toll-like receptor (TLR) 4-driven pro-inflammatory state in the arterial wall, probably resulting from the accretion of SFA (the substrate of SCD1) in infiltrated macrophages [8]. Interestingly, one of the pleiotropic anti-atherosclerotic mechanisms of LCn-3PUFA is the inhibition of SFA-induced activation of TLR4 (as reviewed in [27]). Consistent with this notion, addition of fish oil to treatment with antisense SCD1 oligonucleotides overcame accelerated atherosclerosis in a murine model [28]. Therefore, dietary LCn-3PUFA might display a synergistic dual action, by both direct repression of SCD1 and prevention of the undesirable side-effects of such down-regulation.

Our results tackle the notion of whether all unsaturated fatty acids are equal regarding effects on SCD1. Replacement of coconut oil (a source of SFA) by either olive oil (a source of MUFA) or sunflower oil (a source of n-6 PUFA) for 4 weeks down-regulated SCD1 gene expression in the visceral adipose tissue of rats, which translated into decreased SCD1 activity, as estimated by fatty acid ratios [29]. A similar conclusion was drawn from randomized feeding studies examining the effects on SCD1 of SFA vs. MUFA [14], vs. n-6 PUFA [13], or vs. rapeseed oil (a source of MUFA, C18:2n-6 and C18:3n-3) [12]. Additionally, increasing the ratio of PUFA to SFA during 6 weeks was shown to down-regulate the plasma SCD1 index [11]. These overall results reinforced the link between SCD1 and PUFA, but did not allow discriminating on the effect of different types of unsaturated fats. Thus far, such notion has only been tested in a crossover trial involving customized isocaloric diets containing different types of unsaturated fats, whereby marine n-3 PUFA induced a higher reduction of plasma SCD1 index compared to n-6 PUFA (mostly linoleic) (23% vs. 14%, respectively, P = 0.041) [17]. We searched for dietary determinants of changes in the plasma SCD1 index after pooling subjects enrolled on a trial

comprising supplementation with olive oil (a source of MUFA), mixed nuts (a source of MUFA and PUFA) or an advice to follow a low-fat diet. When including simultaneously all types of dietary fat in the models, we found an association of reduced plasma SCD1 index with dietary long chain (≥20 carbons) PUFA, but not with their shorter-chain counterparts (C18:2n-6 + C18:3n-3) or MUFA, the fats mostly supplied by supplemental foods in the trial. This finding is consistent with data from experimental models suggesting that the level of hepatic SCD1 suppression obtained by PUFA relates to the fatty acid length and degree of unsaturation [10].

Our study has limitations. First, plasma VLDL-TG C16:1n-7/C16:0 is the best surrogate of SCD1 gene expression in the liver [3]. Given the selective C16:1n-7 and C16:0 partitioning among different plasma lipid fractions, the validity of the SCD1 index assessed in whole plasma is compromised when comparing groups with different TG concentrations [24]. To circumvent the artefact that the desaturation index reflects the TG fatty acid profile rather than the actual SCD1 activity, we (i) excluded hypertriglyceridemic subjects from the study; (ii) included changes in fasting plasma TG as a confounder in the multivariate models; and (iii) introduced a statistical adjustment for the confounding effect of altered HDL-cholesterol concentrations, accounting for the possibility that the PREDIMED intervention could also have resulted in altered lipoprotein concentrations, thereby influencing the whole plasma fatty acid composition due to their relative richness in phospholipids. Second, as recently stated [3], the C16:1n-7/C16:0 and C18:1n-9/C18:0 ratios are not necessarily interchangeable as indirect measures of the plasma SCD1 index. We avoided the use of the C18:1n-9/C18:0 ratio as a surrogate marker of the plasma SCD1 index because the proportion of C18:1n-9 in plasma might be

enhanced by increased olive oil consumption in our population and, importantly, because it does not allow to discriminate between endogenous and exogenous supplies of this fatty acid. In contrast, C16:1n-7 is an optimal marker of endogenous MUFA synthesis in our study subjects, since C16:1n-7 is supplied by few foods (i.e. macadamia nuts), which are uncommonly consumed in Spain [30]. Finally, because the study was conducted in a specific population at high risk for cardiovascular disease, results cannot be easily extrapolated to healthy populations.

In conclusion, in a 1-year study conducted in non-hypertriglyceridemic subjects at high cardiovascular risk, we found that increasing consumption of LCn-3PUFA (resulting from fatty fish consumption with the usual diet) but not intake of plant-derived MUFA or PUFA, was associated with reduced plasma SCD1 index after adjustment for several confounders, including changes in fasting TG. Our results provide clinical evidence of interplay between customary omega-3 intake, SCD1 and adiposity, thus far a barely explored topic in humans.

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Statement of authorship

The author's responsibilities were as follows: JS-S, MAM-G, CL-S, DC, ER and RE secured funding; JM-P performed the gas-chromatographic analyses; ER and AS-V prepared the manuscript and conducted the statistical analyses, with important input and feedback from all coauthors; MAM-G and JS-S participated in the design and execution of the study and contributed to the critical revision of the manuscript for important intellectual content. All authors read and approved the final version to be submitted.

Conflict of interest statement

JS-S has received research funding from the International Nut Council, Reus, Spain. He is a non-paid member of the Scientific Advisory Board of the International Nut Council. ER has received research funding (included unrestricted grants) from the California Walnut Commission, Sacramento, CA and is a non-paid member of its Scientific Advisory Committee. The authors have no other conflict of interest to declare.

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REFERENCES

[1]. Yilmaz M, Claiborn KC, Hotamisligil GS. De Novo Lipogenesis Products and Endogenous Lipokines. Diabetes. 2016;65:1800–1807.

[2]. Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. Am. J. Physiol. Endocrinol. Metab. 2009;297:E28–E37.

[3]. Hodson L, Fielding BA. Stearoyl-CoA desaturase: rogue or innocent bystander? Prog. Lipid Res. 2012;52:15–42.

[4]. Warensjö E, Risérus U, Vessby B. Fatty acid composition of serum lipids predicts the development of the metabolic syndrome in men. Diabetologia. 2005;48:1999–2005.

[5]. Vinknes KJ, Elshorbagy AK, Nurk E, et al. Plasma stearoyl-CoA desaturase indices: association with lifestyle, diet, and body composition. Obesity. 2013;21:E294–E302.

[6]. Puri P, Wiest MM, Cheung O, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. Hepatology. 2009;50:1827–1838.

[7]. Warensjö E, Sundström J, Vessby B, et al. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population-based prospective study. Am. J. Clin. Nutr. 2008;88:203–209.

[8]. Brown JM, Chung S, Sawyer JK, et al. Inhibition of stearoyl-coenzyme A desaturase 1 dissociates insulin resistance and obesity from atherosclerosis. Circulation. 2008;118:1467–1475.

[9]. Chong MFF, Hodson L, Bickerton AS, et al. Parallel activation of *de novo* lipogenesis and stearoyl-CoA desaturase activity after 3 d of high-carbohydrate feeding. Am. J. Clin. Nutr. 2008;87:817–823.

[10]. Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid. Res. 1999;40:1549–1558.

[11]. Velliquette RA, Gillies PJ, Kris-Etherton PM, et al. Regulation of human stearoyl-CoA desaturase by omega-3 and omega-6 fatty acids: Implications for the dietary management of elevated serum triglycerides. J. Clin. Lipidol. 2009;3:281–288.

[12]. Warensjö E, Risérus U, Gustafsson IB, et al. Effects of saturated and unsaturated fatty acids on estimated desaturase activities during a controlled dietary intervention. Nutr. Metab. Cardiovasc. Dis. 2008;18:683–690.

[13]. Bjermo H, Iggman D, Kullberg J, et al. Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. Am. J. Clin. Nutr. 2012;95:1003–1012.

[14]. Gebauer SK, West SG, Kay CD, et al. Effects of pistachios on cardiovascular disease risk factors and potential mechanisms of action: a dose-response study. Am. J. Clin. Nutr. 2008;88:651–659.

[15]. Vessby B, Gustafsson IB, Tengblad S, et al. Indices of fatty acid desaturase activity in healthy human subjects: effects of different types of dietary fat. Br. J. Nutr. 2013;110:871–879.

[16]. Telle-Hansen VH, Larsen LN, Høstmark AT, et al. Daily intake of cod or salmon for 2 weeks decreases the 18:1n-9/18:0 ratio and serum triacylglycerols in

healthy subjects. Lipids 2012;47:151–160. Erratum in: Lipids 2012; 47:755–756.

[17]. Karlström BE, Järvi AE, Byberg L, et al. Fatty fish in the diet of patients with type 2 diabetes: comparison of the metabolic effects of foods rich in n-3 and n-6 fatty acids. Am. J. Clin. Nutr. 2011;94:26–33.

[18]. Estruch R, Ros E, Salas-Salvadó J, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N. Engl. J. Med. 2013;368:1279–1290. Erratum in: N. Engl. J. Med. 2014;370:886.

[19]. Elosua R, Garcia M, Aguilar A, et al. Validation of the Minnesota Leisure Time Physical Activity Questionnaire in Spanish women. Investigators of the MARATDOM Group. Med. Sci. Sports Exerc. 2000;32:1431–1437.

[20]. Fernández-Ballart JD, Piñol JL, Zazpe I, et al. Relative validity of a semi-quantitative food-frequency questionnaire in an elderly Mediterranean population of Spain. Br. J. Nutr. 2010;103:1808–1816.

[21]. Moreiras O, Carbajal A, Cabrera L, et al. Tablas de Composición de Alimentos (Food Composition Tables). Madrid: Ediciones Pirámide, S.A 2005.

[22]. Schröder H, Fitó M, Estruch R, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr. 2011 Jun;141(6):1140–1145.

[23]. Bondia-Pons I, Castellote AI, López-Sabater MC. Comparison of conventional and fast gas chromatography in human plasma fatty acid determination. J. Chromatogr. B 2004;809:339–344.

[24]. Karpe F, Hodson L. Caution on the interpretation of plasma fatty acid composition as a proxy marker for SCD1 activity: particular implications for

using the 16:1/16:0 ratio in QTL studies involving hyperlipidemic patients. Arterioscler. Thromb. Vasc. Biol. 2008 28:e152.

[25]. Martínez-Fernández L, Laiglesia LM, Huerta AE, Martínez JA, Moreno-Aliaga MJ. Omega-3 fatty acids and adipose tissue function in obesity and metabolic syndrome. Prostaglandins Other Lipid Mediat. 2015;121:24–41.

[26]. Kris-Etherton PM, Harris WS, Appel LJ; American Heart Association. Nutrition Committee. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106:2747–2757.

[27]. Adkins Y, Kelley DS. Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids. J. Nutr. Biochem. 2010;21:781-792.

[28]. Brown JM, Chung S, Sawyer JK, et al. Combined therapy of dietary fish oil and stearoyl-CoA desaturase 1 inhibition prevents the metabolic syndrome and atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 2010;30:24–30.

[29]. Girón MD, Lara A, Suárez MD. Short-term effects of dietary fats on the lipid composition and desaturase activities of rat liver microsomes. Biochem. Mol. Biol. Int. 1996;40:843–851.

[30]. Aranceta J, Pérez-Rodrigo C, Naska A, et al. Nut consumption in Spain and other countries. Br. J. Nutr. 2006;96:3S-11S. Erratum in: Br. J. Nutr. 2008;99:447–448.

Table 1. Baseline clinical characteristics, lipid profiles, and medication use in the whole sample and in selected non-hypertriglyceridemic subjects at high vascular risk.

Variable	Whole sample (n = 391)	Non-hypertrigliceridemic subjects (n = 243)	P *
Triglycerides, mg/dL	127 (121 to 133)	97 (94 to 101)	<0.001
Male, n (%)	164 (41.9)	105 (43.2)	0.754
Age, years	67.7 (67.1 to 68.3)	68.0 (67.3 to 68.8)	0.480
Family history of early-onset CHD, n (%) †	80 (21.7)	48 (21.2)	0.899
Weight, kg	74.3 (73.3 to 75.3)	73.8 (72.5 to 75.1)	0.539
Body mass index, kg/m^2	29.3 (29.0 to 29.6)	29.0 (28.6 to 29.4)	0.159
Waist circumference, cm	99 (98 to 100)	98 (97 to 99)	0.333
Leisure-time physical activity, MET-min/d	253 (231 to 275)	265 (237 to 293)	0.491
Current smoker, n (%)	57 (14.6)	33 (13.6)	0.726
Former smoker, n (%)	84 (21.5)	54 (22.2)	0.827
Dyslipidemia, n (%)	267 (68.3)	164 (67.5)	0.834
Use of statins, n (%)	160 (40.9)	98 (40.3)	0.883
Total cholesterol, mg/dL	211 (207 to 215)	209 (205 to 214)	0.565
HDL cholesterol, mg/dL	56 (55 to 58)	59 (57 to 61)	0.030
Non-HDL cholesterol, mg/dL	155 (151 to 158)	150 (146 to 155)	0.136
Hypertension, n (%)	327 (83.6)	200 (82.3)	0.664
Use of antihypertensive agents, n (%)	278 (71.1)	163 (67.1)	0.285
Fasting glucose, mg/dL	117 (113 to 120)	112 (108 to 117)	0.139
Type 2 diabetes, n (%)	173 (44.2)	104 (42.8)	0.721
Use of insulin, n (%)	22 (5.6)	14 (5.8)	0.943
Use of oral antihyperglycemic drugs, n (%)	100 (25.6)	56 (23.0)	0.472

Values are n (%), except for age, weight, body mass index, and physical activity, expressed as means (95% CI).
CHD, coronary heart disease; MET-min, minutes at a given metabolic equivalent level (units of energy expenditure in physical activity, 1 MET-min is roughly equivalent to 1 kcal).
* Obtained by the chi-square test or ANOVA, as appropriate.
† Data from n = 369 / n = 226 participants.

Table 2. Baseline intake and 1-year changes in intake of nutrients and selected foods in 243 non-hypertriglyceridemic subjects at high vascular risk.

Variable		Values	Range	P *
Energy (Kcal/d)	Baseline	2144 (490)	1168 to 3837	0.001
	Change	129 (593)	-1671 to 2119	
Protein (g/d)	Baseline	91 (19)	49 to 165	<0.001
	Change	5 (24)	-58 to 90	
Total carbohydrate (g/d)	Baseline	221 (66)	105 to 516	<0.001
	Change	7 (79)	-231 to 233	
Total fat (g/d)	Baseline	92 (24)	38 to 174	<0.001
	Change	10 (32)	-66 to 123	
SFA (g/d)	Baseline	24 (8)	8 to 48	<0.001
	Change	1 (9)	-21 to 39	
MUFA (g/d)	Baseline	46 (13)	17 to 88	<0.001
	Change	5 (17)	-34 to 63	
Plant-derived PUFA (g/d) †	Baseline	14 (5)	5 to 38	<0.001
	Change	3 (8)	-22 to 32	
LCn-3PUFA (g/d) ‡	Baseline	0.9 (0.5)	0.0 to 4.2	0.010
	Change	0.1 (0.6)	-2.4 to 2.6	
Extra-virgin olive oil (g/d)	Baseline	24 (21)	0 to 70	0.016
	Change	8 (24)	-50 to 70	
Other types of olive oil (g/d) ¶	Baseline	12 (18)	0 to 70	<0.001
	Change	-4 (16)	-70 to 50	
Other vegetable oils (g/d) §	Baseline	2 (5)	0 to 25	0.002
	Change	1 (4)	0 to 50	
Nuts (g/d)	Baseline	11 (14)	0 to 75	<0.001
	Change	6 (12)	-51 to 30	
Fatty fish (g/d)	Baseline	27 (22)	0 to 130	0.136
	Change	3 (27)	-74 to 130	

Values are means (SD). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. LCn-3PUFA, long-chain n-3 polyunsaturated fatty acids.
* Obtained by paired-test between baseline and 1-y value, adjusting for 1-y differences in total energy intake.
† C18:2n-6 + C18:3n-3.
‡ C20:5n-3 + C22:6n-3.
¶ Refined and pomace olive oil.

§ Sum of intakes of sunflower, corn and soybean oil.

Table 3. Independent determinants of 1-y changes in adiposity traits in 243 non-hypertriglyceridemic subjects at high vascular risk.

Independent variable	Weight, kg		Body mass index, kg/m^2		Waist, cm	
	B (95% CI)	P	B (95% CI)	P	B (95% CI)	P
Baseline value	-0.034 (-0.075 to 0.006)	0.098	-0.039 (-1.645 to 0.016)	0.162	-0.205 (-0.285 to -0.125)	<0.001
Sex, male	-1.021 (-1.861 to -0.181)	0.017	-0.213 (-0.564 to 0.137)	0.232	-1.737 (-3.259 to -0.215)	0.025
Age, y	0.008 (-0.060 to 0.075)	0.824	0.009 (-0.020 to 0.038)	0.536	0.107 (-0.018 to 0.232)	0.093
1-y changes in physical activity, MET-min/d	0.000 (-0.001 to 0.001)	0.970	0.000 (-0.001 to 0.001)	0.915	0.000 (-0.003 to 0.003)	0.919
1-y changes in total energy intake, 100 Kcal/d	-0.045 (-0.112 to 0.021)	0.182	-0.020 (-0.049 to 0.009)	0.182	0.071 (-0.054 to 0.195)	0.265
1-y changes in fasting HDL cholesterol, mg/dL	0.003 (-0.041 to 0.046)	0.902	0.006 (-0.013 to 0.025)	0.532	0.016 (-0.065 to 0.097)	0.703
1-y changes in fasting triglycerides, mg/dL	-0.008 (-0.023 to 0.007)	0.290	-0.004 (-0.010 to 0.003)	0.290	0.009 (-0.019 to 0.038)	0.516
1-y changes in plasma SCD1 index $\times 10^2$	0.221 (0.021 to 0.422)	0.031	0.115 (0.027 to 0.202)	0.011	-0.116 (-0.491 to 0.258)	0.542
Interaction between change in plasma SCD1 index $\times$ change in fasting triglycerides	-0.003 (-0.011 to 0.004)	0.388	-0.001 (-0.005 to 0.002)	0.399	-0.031 (-0.045 to -0.017)	<0.001

Data are presented as B (non-standardized regression coefficient) with 95% confidence interval for the stated increase in independent variables.

Table 4. Dietary (nutrients) determinants of 1-y changes in plasma SCD1 index in 243 non-hypertriglyceridemic subjects at high vascular risk, by multivariate linear regression.

	Model 1		**Model 2**	
	B (95% CI)	P	B (95% CI)	P
Energy, 100 kcal/d	-0.115 (-0.847 to 0.617)	0.757	-0.110 (-0.841 to 0.622)	0.768
Protein, 5 g/d	0.041 (-0.166 to 0.249)	0.694	0.042 (-0.165 to 0.249)	0.689
Total carbohydrates, 10 g/d	0.045 (-0.248 to 0.339)	0.762	0.043 (-0.251 to 0.336)	0.776
Cholesterol, 50 mg/d	0.037 (-0.122 to 0.197)	0.647	0.029 (-0.132 to 0.189)	0.725
SFA, 5 g/d	0.036 (-0.334 to 0.405)	0.850	0.034 (-0.335 to 0.403)	0.856
MUFA, 5 g/d	0.111 (-0.278 to 0.501)	0.574	0.108 (-0.282 to 0.497)	0.586
Plant-derived PUFA, 5 g/d *	-0.009 (-0.092 to 0.075)	0.839	-0.010 (-0.093 to 0.074)	0.821
LCn-3PUFA, 1g/d †	-0.562 (-1.062 to -0.063)	0.027	-0.544 (-1.044 to -0.043)	0.033
Alcohol, 5 g/d	0.030 (-0.254 to 0.314)	0.834	0.027 (-0.258 to 0.311)	0.854
Interaction between change in LCn-3PUFA × change in fasting triglycerides	--	--	-0.008 (-0.024 to 0.007)	0.288

Data are presented as B (non-standardized regression coefficient) with 95% confidence intervals for the stated 1-y increase in each nutrient. Sex, age, 1-year changes in fasting triglycerides, 1-year changes in fasting HDL cholesterol, and 1-year changes in physical activity were also included as potential confounders.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.

* C18:2n-6 + C18:3n-3.
† C20:5n-3 + C22:6n-3.

Table 5. Dietary (food groups) determinants of 1-y changes in plasma SCD1 index in 243 non-hypertriglyceridemic subjects at high vascular risk, by multivariate linear regression.

	Model 1		**Model 2**	
	B (95% CI)	P	B (95% CI)	P
Vegetables, 50 g/d	-0.010 (-0.104 to 0.083)	0.826	-0.015 (-0.109 to 0.078)	0.747
Fruit, 50 g/d	0.013 (-0.053 to 0.079)	0.701	0.011 (-0.056 to 0.077)	0.751
Pulses, 5 g/d	-0.039 (-0.172 to 0.095)	0.568	-0.043 (-0.176 to 0.090)	0.528
Cereals, 50 g/d	-0.008 (-0.123 to 0.108)	0.895	-0.001 (-0.116 to 0.115)	0.992
Dairy products, 50 g/d	0.029 (-0.043 to 0.102)	0.423	0.032 (-0.040 to 0.105)	0.382
Meat and processed meat, 25 g/d	0.145 (-0.074 to 0.364)	0.193	0.151 (-0.069 to 0.370)	0.177
Added sugars, 5 g/d	-0.048 (-0.119 to 0.023)	0.185	-0.054 (-0.126 to 0.017)	0.137
Wine, 25 mL/d	0.025 (-0.048 to 0.097)	0.503	0.024 (-0.049 to 0.096)	0.521
Lean fish, 10 g/d	-0.054 (-0.142 to 0.034)	0.230	-0.055 (-0.143 to 0.033)	0.217
Fatty fish, 10 g/d	-0.084 (-0.178 to 0.011)	0.083	-0.083 (-0.177 to 0.012)	0.085
Interaction between change in fatty fish × change in fasting triglycerides	--	--	-0.002 (-0.006 to 0.001)	0.215

Data are presented as B (non-standardized regression coefficient) with 95% confidence intervals for the stated 1-y increase in each food group. Sex, age, 1-year changes in fasting triglycerides, 1-year changes in fasting HDL cholesterol, and 1-year changes in physical activity were also included as potential confounders.

HIGHLIGHTS

- There is increasing interest on the interplay between SCD1, PUFA and adiposity
- We analysed 1-y changes of these variables in non-hypertriglyceridemic elders
- 1-y changes in plasma SCD1 directly related to weight and BMI changes
- Changes in dietary LCn-3PUFA inversely related to plasma SCD1 index
- The results reinforce the evidence of marine n-3 fatty acids as healthy fats