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FADS1 Genetic Variability Interacts with Dietary α-Linolenic Acid Intake to Affect Serum Non-HDL–Cholesterol Concentrations in European Adolescents[1–3]

Julie Dumont,[4] Inge Huybrechts,[5] Andre Spinneker,[6] Frédéric Gottrand,[7] Evangelia Grammatikaki,[8] Noemi Bevilacqua,[9] Krishna Vyncke,[5] Kurt Widhalm,[10] Anthony Kafatos,[11] Denes Molnar,[12] Idoia Labayen,[13,14] Marcela Gonzalez-Gross,[15] Philippe Amouyel,[4] Luis A. Moreno,[16] Aline Meirhaeghe,[4] and Jean Dallongeville,[4]* on behalf of the HELENA Study group[17]

[4]Inserm, U744, Institut Pasteur de Lille, Univ Lille Nord de France, UDSL, Lille 59000, France; [5]Department of Public Health, Faculty of Medicine and Health Sciences, Ghent University, Ghent 900, Belgium; [6]Institut für Ernährungs- und Lebensmittelwissenschaften – Humanernährung, Rheinische Friedrich-Wilhelms Universität, Bonn 53115, Germany; [7]Inserm, U995, Faculté de Médecine, Univ Lille Nord de France, UDSL, Lille 59000, France; [8]Department of Nutrition and Dietetics, Harokopio University, Athens, Greece; [9]National Institute for Food and Nutrition, Rome 00118, Italy; [10]Department of Pediatrics, Division of Clinical Nutrition and Prevention, Medical University of Vienna, Vienna A-1090, Austria; [11]University of Crete School of Medicine, PO. 1393 Greece; [12]University of Pècs, Department of Paediatrics, Pècs H-7624, Hungary; [13]Department of Nutrition and Food Science, University of the Basque Country, Vitoria 01006, Spain; [14]Unit for Preventive Nutrition, Department of Biosciences and Nutrition at NOVUM, Karolinska Institutet, SE-17177 Huddinge, Sweden; [15]Department of Health and Human Performance, Faculty of Physical Activity and Sport Sciences, INEF, Universidad Politécnica de Madrid, Madrid 28002, Spain; and [16]GENUD Research Group, Escuela Universitaria de Ciencias de la Salud, Universidad de Zaragoza, Zaragoza 50009, Spain

Abstract

Two rate-limiting enzymes in PUFA biosynthesis, Δ5- and Δ6-desaturases, are encoded by the *FADS1* and *FADS2* genes, respectively. Genetic variants in the *FADS1-FADS2* gene cluster are associated with changes in plasma concentrations of PUFA, HDL- and LDL-cholesterol, and TG. However, little is known about whether dietary PUFA intake modulates these associations, especially in adolescents. We assessed whether dietary linoleic acid (LA) or α-linolenic acid (ALA) modulate the association between the *FADS1* rs174546 polymorphism and concentrations of PUFA, other lipids, and lipoproteins in adolescents. Dietary intakes of LA and ALA, *FADS1* rs174546 genotypes, PUFA levels in serum phospholipids, and serum concentrations of TG, cholesterol, and lipoproteins were determined in 573 European adolescents from the HELENA study. The sample was stratified according to the median dietary LA (≤9.4 and >9.4 g/d) and ALA (≤1.4 and >1.4 g/d) intakes. The associations between *FADS1* rs174546 and concentrations of PUFA, TG, cholesterol, and lipoproteins were not affected by dietary LA intake (all *P*-interaction > 0.05). Similarly, the association between the *FADS1* rs174546 polymorphism and serum phospholipid concentrations of ALA or EPA was not modified by dietary ALA intake (all *P*-interaction > 0.05). In contrast, the rs174546 minor allele was associated with lower total cholesterol concentrations ($P = 0.01$ under the dominant model) and non-HDL–cholesterol concentrations ($P = 0.02$ under the dominant model) in the high-ALA–intake group but not in the low-ALA–intake group (*P*-interaction = 0.01). These results suggest that dietary ALA intake modulates the association between *FADS1* rs174546 and serum total and non-HDL–cholesterol concentrations at a young age. J. Nutr. 141: 1247–1253, 2011.

Introduction

PUFA concentrations are determined by dietary intake and endogenous synthesis via the successive elongation and desaturation of the dietary precursors linoleic-acid [LA;[18] 18:2(n-6)] and α-linolenic acid [ALA; 18:3(n-3)]. Δ5-Desaturase (D5D) and Δ6-desaturase are required for the synthesis of long-chain PUFA (LC-PUFA) in mammals (1) and are encoded by the *FADS1* and *FADS2* genes, respectively (located in a cluster on chromosome 11q12–13.1). Several studies have reported strong

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* To whom correspondence should be addressed. E-mail: jean.dallongeville@pasteur-lille.fr.

[18] Abbreviations used: ALA, α-linolenic acid; ARA, arachidonic acid; D5D, Δ5-desaturase; FA, fatty acid; HELENA Study, Healthy Lifestyle in Europe by Nutrition in Adolescence; HELENA-DIAT, HELENA Dietary Assessment Tool; LA, linoleic acid; LC-PUFA, long-chain PUFA; MSM, Multiple Source Method; SNP, single nucleotide polymorphism.

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associations between single nucleotide polymorphisms (SNPs) in the *FADS1-FADS2* gene cluster and PUFA concentrations in adipose tissue, plasma, or erythrocytes in both adults (2–8) and adolescents (HELENA study) (9).

Other studies have reported relationships between several *FADS1-FADS2* variants and plasma lipid concentrations (2,3,10–13). In a recent meta-analysis, the minor allele of the *FADS1* rs174546 polymorphism was associated with lower concentrations of HDL- and LDL-cholesterol and higher TG concentrations in men and women of European ancestry (14). In contrast, in the HELENA study, the rs174546 polymorphism was not associated with serum lipid and lipoprotein concentrations in European adolescents (9). There are several explanations for the divergent results in adults and adolescents. First, the discrepancy might be due to a lack of statistical power in the HELENA study. Indeed, based on the effect sizes reported by Teslovich et al. (14) and with a minor allele frequency of 0.32 (such as for rs174546), the statistical power values to detect an association ($P < 0.05$) with total cholesterol, HDL-cholesterol, LDL-cholesterol, and TG were 30, 9, 34, and 72%, respectively. Second, the changes in plasma lipid/lipoprotein profile that occur during the transition from childhood to adolescence (15) may mask the association between *FADS1* polymorphism and lipid concentrations in the young. Alternatively, dietary habits, which may differ between adults and adolescents, could confound the association between *FADS1-FADS2* genetic variants and lipid concentrations. In agreement with this hypothesis, Lu et al. (16) recently reported that the association between rs174546 and cholesterol concentrations is modulated by the dietary intake of (n-3) and (n-6) PUFA in adults. Therefore, the goal of the present study was to determine whether the dietary intakes of LA and/or ALA influenced the association between *FADS1* rs174546 and serum lipid and lipoprotein concentrations in European adolescents.

Methods

The HELENA study. The recruitment and phenotyping of the adolescents participating in the HELENA cross-sectional study have been described previously (17). Briefly, a total of 3865 adolescents (age range: 12.5–17.5 y) were recruited between 2006 and 2007. Data were collected in a total of 10 centers in 9 European countries. Participants were randomly selected from schools by using a proportional cluster sampling methodology and taking age into account. One-third of the classes were randomly selected for blood collection; this resulted in a total of 1155 blood samples.

Medical history, medications used, and any information related to medical examinations were recorded in a specific case report form for each participant in accordance with standardized procedures (18). In each center, trained researchers carried out complete physical examinations, including weight, height, and blood pressure measurements. The protocol was approved by the appropriate investigational review board for each investigating center. The study was conducted in accordance with the Helsinki Declaration and was approved by independent ethical committees (19). Written, informed consent was obtained from each adolescent and both of his/her parents or legal representatives. Participation was voluntary.

From the initial sample (n = 1144), the HELENA data management group excluded a priori 2 participating centers with missing data on dietary intake [Crete (n = 110) and Hungary (n = 137)], 175 adolescents with incomplete dietary recalls, and 149 under-reporters (for methodology, see the "Dietary intake assessment" section). This resulted in a sample of 573 adolescents with fully available genetic, biochemical, and dietary data (2 valid 24-h dietary recalls) (20). Main characteristics of included and excluded participants are presented in **Supplemental Table 1**.

Biochemical measurements. Venous blood samples were drawn after a 10-h overnight fast and sent to a central laboratory (the Analytical Laboratory at the University of Bonn's Institut für Ernährungs- und Lebensmittelwissenschaften). Serum TG and HDL- and LDL-cholesterol concentrations were enzymatically assayed on the Dimension RxL clinical chemistry system (Dade Behring).

After Folch extraction of serum samples, the phospholipid fraction was separated using TLC. The phospholipid band was scraped off and the fatty acids (FA) were converted into their methyl esters by transesterification with methanol/hydrochloric acid. The phospholipid fraction's FAME were analyzed using GC (Model 3900, Varian) on a 30-m × 0.25-mm × 0.25-μm polyethylene glycol column (Zebron ZB-WAXplus, Phenomenex). Peaks of interest were identified by comparison with authentic FAME standards (Sigma-Aldrich). FA were expressed as a percentage area by integrating the area under the peak and dividing it by the total area for all FA. The CV were <4.4% for all FA analyses.

SNP selection and genotyping. Blood for DNA extraction was collected in EDTA K3 tubes, stored at the Institut für Ernährungs- und Lebensmittelwissenschaften, and then sent to the Genomic Analysis Laboratory at the Institut Pasteur de Lille. DNA was extracted from white blood cells with the Puregene kit (QIAGEN) and stored at −20°C.

To test the interaction between PUFA intake and *FADS* gene cluster polymorphisms and serum lipid concentrations, the *FADS1* rs174546 polymorphism was selected on the basis of the following criteria: *1*) the strongest associations with serum phospholipid LC-PUFA have been observed for this polymorphism in the HELENA study (9); *2*) it is a tag-SNP for the cluster of polymorphisms found to be associated with plasma lipid concentrations in a recent meta-analysis of genome-wide association studies (14); and *3*) the rs174546 polymorphism is the only one known to interact with dietary PUFA intake to affect plasma cholesterol concentrations in adults (16). Adolescents were genotyped for *FADS1* rs174546 on an Illumina system using GoldenGate technology. The genotyping success rate was 99.8%.

[2] Author disclosures: J. Dumont, I. Huybrechts, A. Spinneker, F. Gottrand, E. Grammatikaki, N. Bevilacqua, K. Vyncke, K. Widhalm, A. Kafatos, D. Molnar, I. Labayen, M. Gonzalez-Gross, P. Amouyel, L. A. Moreno, A. Meirhaeghe, and J. Dallongeville, no conflicts of interest.

[3] Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.

[17] HELENA Study Group Coordinator: Luis A. Moreno. Core group members: Luis A. Moreno, Fréderic Gottrand, Stefaan De Henauw, Marcela González-Gross, Chantal Gilbert. Steering committee: Anthony Kafatos (President), Luis A. Moreno, Christian Libersa, Stefaan De Henauw, Sara Castelló, Fréderic Gottrand, Mathilde Kersting, Michael Sjöstrom, Dénes Molnár, Marcela González-Gross, Jean Dallongeville, Chantal Gilbert, Gunnar Hall, Lea Maes, and Luca Scalfi. Project Manager: Pilar Meléndez. 1. Universidad de Zaragoza (Spain) Luis A. Moreno, Jesús Fleta, José A. Casajús, Gerardo Rodríguez, Concepción Tomás, María I. Mesana, Germán Vicente-Rodríguez, Adoración Villarroya, Carlos M. Gil, Ignacio Ara, Juan Revenga, Carmen Lachen, Juan Fernández Alvira, Gloria Bueno, Aurora Lázaro, Olga Bueno, Juan F. León, Jesús Mª Garagorri, Manuel Bueno, Juan Pablo Rey López, Iris Iglesia, Paula Velasco, Silvia Bel, and Theodora Mouratidou. 2. Consejo Superior de Investigaciones Científicas (Spain) Ascensión Marcos, Julia Wärnberg, Esther Nova, Sonia Gómez, Ligia Esperanza Díaz, Javier Romeo, Ana Veses, Belén Zapatera, Tamara Pozo, and David Martínez. 3. Université de Lille 2 (France) Laurent Beghin, Christian Libersa, Frédéric Gottrand, Catalina Iliescu, and Juliana Von Berlepsch. 4. Research Institute of Child Nutrition Dortmund, Rheinische Friedrich-Wilhelms-Universität Bonn (Germany) Mathilde Kersting, Wolfgang Sichert-Hellert, and Ellen Koeppen. 5. Pécsi Tudományegyetem (University of Pécs) (Hungary) Dénes Molnar, Eva Erhardt, Katalin Csernus, Katalin Török, Szilvia Bokor, Mrs. Angster, Enikö Nagy, Orsolya Kovács, and Judit Répasi. 6. University of Crete School of Medicine (Greece) Anthony Kafatos, Caroline Codrington, María Plada, Angeliki Papadaki, Katerina Sarri, Anna Viskadourou, Christos Hatzis, Michael Kiriakakis, George Tsibinos, Constantine Vardavas, Manolis Sbokos, Eva Protoyeraki, and Maria Fasoulaki. 7. Institut für Ernährungs- und Lebensmittelwissenschaften – Ernährungphysiologie. Rheinische Friedrich Wilhelms Universität, Bonn (Germany) Peter Stehle, Klaus Pietrzik, Marcela González-Gross, Christina Breidenassel, Andre Spinneker, Jasmin Al-Tahan, Miriam Segoviano, Anke Berchtold, Christine Bierschbach, Erika Blatzheim, Adelheid Schuch, and Petra Pickert. 8. University of Granada (Spain) Manuel J. Castillo, Ángel Gutiérrez, Francisco B Ortega, Jonatan R Ruiz, Enrique G Artero, Vanesa España, David Jiménez-Pavón, Palma Chillón, Cristóbal Sánchez-Muñoz, and Magdalena Cuenca. 9. Istituto Nazionalen di Ricerca per gli

Dietary intake assessment. Following the recommendations of the European Food Consumption Survey Method project (21), the adolescents completed 2 nonconsecutive, self-reported, 24-h recalls by using the computer-based HELENA Dietary Assessment Tool (HELENA-DIAT). This tool is based on recalling 6 meal occasions from the day before the interview. The adolescents completed the questionnaire during school time after dieticians and researchers had instructed them on how to perform 24-h recalls as accurately as possible. The participants were allowed to ask questions and request assistance. After completion, the recall was checked for completeness. Each participant was asked to fill out the HELENA-DIAT on 2 arbitrary days within a 2-wk period. Because the questionnaire was completed during school time (Monday to Friday), data on dietary intake on Fridays and Saturdays could not be collected. A validation study by Vereecken et al. (20) has demonstrated that the self-reported YANA-C (a previous version of the HELENA-DIAT) agreed well with an interviewer-administered version of the YANA-C. The HELENA-DIAT tool has been described as a good method for collecting detailed dietary information from adolescents and was well received by the study participants (22).

To calculate energy and nutrient intakes, data from the HELENA-DIAT was linked to the German Food Code and Nutrient Database (Bundeslebensmittelschlüssel, version II.3.1, 2005). The usual dietary intake of nutrients and foods (including episodically consumed foods) was estimated by applying the Multiple Source Method (MSM) (23). The MSM calculates dietary intake for individuals first and then constructs a population distribution based on the individual data. In the present study, the MSM was used to correct dietary data for between- and within-person variability. Under-reporters were excluded from all analyses. The basal metabolic rate was calculated from age-specific FAO/WHO/UNU equations (24). Under-reporting was considered when the ratio of energy intake:estimated basal metabolic rate was <0.96, as proposed by Black (25).

Statistical methods. Statistical analyses were performed with SAS software (SAS Institute). Departure from Hardy-Weinberg equilibrium within the study groups was evaluated using a χ^2 test. Dietary LA and ALA intakes were classified a priori into 2 groups according to the study population median intake: ≤9.4 and >9.4 g/d for LA and ≤1.4 and >1.4 g/d for ALA. Prior to analysis, intake values were normalized by log-transformation. The strength of association between dietary LA and ALA intakes and their corresponding serum phospholipids concentrations was evaluated by estimating the Pearson correlation coefficient (r). Due to the limited sample size, especially after stratification for dietary LA and ALA intakes, the number of participants homozygous for the minor allele in each subgroup was low ($29 \leq n \leq 36$), resulting in a high variability of the relevant traits and an inequality of variance between genotypes. For this reason, analyses were performed using a dominant model. Sensitivity analyses were carried out using an additive model. To analyze the association between *FADS1* genotypes and PUFA, lipid or lipoprotein concentrations, we relied on a multilevel random regression analysis using a mixed linear model. This multilevel analysis allows to take the variability associated with the school within center sampling strategy into account. The interactions between dichotomized LA and ALA intakes and genotypes on quantitative variables were explored by including additional interaction terms in the mixed linear model. All tests were adjusted for age, gender, BMI, and total energy intake. $P < 0.05$ was considered significant. Power calculations were performed using Quanto v1.2.4 (26). The statistical power to detect an association ($P < 0.05$) was estimated on the basis of the effect sizes reported in a recent meta-analysis of genome-wide association studies (14) and with a minor allele frequency of 32%.

Results

The *FADS1* rs174546 genotype distribution fulfilled Hardy-Weinberg equilibrium (χ^2 = 1.34; P = 0.24). In the present sample, allele frequency for the minor allele of the rs174546 SNP was 0.32. Consistent with our previous work in a larger sample from the HELENA study (9) and whatever the genetic model tested (i.e. additive or dominant), the minor allele of rs174546 was associated with higher serum phospholipid concentrations of LA ($P < 0.0001$) and ALA ($P \leq 0.0002$) and lower serum phospholipid levels of arachidonic acid (ARA) ($P < 0.0001$) and EPA ($P \leq 0.003$) (**Table 1**). The dietary intakes of LA and ALA did not differ significantly between *FADS1* genotype groups. There were no significant associations between the *FADS1* rs174546 polymorphism and HDL-, non-HDL–, LDL–cholesterol, or TG concentrations (Table 1).

Participants were classified into 2 groups (low and high) according to the median dietary LA and ALA intakes for the study population as a whole (with cutoffs at 9.4 and 1.4 g/d, respectively). The median LA and ALA intakes were 7.5 and 1.1 g/d in the low-intake group and 12.0 and 1.8 g/d in the high-intake group, respectively. Serum phospholipid levels of LA were higher in the high-intake group (22.3 ± 0.2%) than in the low-intake group (21.6 ± 0.2%) (P = 0.01). In contrast, ALA intake was not related to serum phospholipid levels of ALA. No significant associations between dietary LA or ALA intake and any of the lipid or lipoprotein variables were observed (data not shown).

Whatever the genetic model tested, there was no significant interaction between dietary LA intake and the *FADS1* rs174546 polymorphism on serum phospholipid levels of LA or ARA (**Table 2**; **Supplemental Table 2**). In both the low- and high-LA-intake groups, the rs174546 T allele was associated with higher LA concentrations and lower ARA concentrations. Similarly, there was no interaction between LA intake and *FADS1* rs174546 on the concentrations of lipids and lipoproteins. After stratification by LA intake, there was still no association

Alimenti e la Nutrizione (Italy) Davide Arcella, Elena Azzini, Emma Barrison, Noemi Bevilacqua, Pasquale Buonocore, Giovina Catasta, Laura Censi, Donatella Ciarapica, Paola D'Acapito, Marika Ferrari, Myriam Galfo, Cinzia Le Donne, Catherine Leclercq, Giuseppe Maiani, Beatrice Mauro, Lorenza Mistura, Antonella Pasquali, Raffaela Piccinelli, Angela Polito, Raffaella Spada, Stefania Sette, and Maria Zaccaria. 10. Dept of Food Science, University of Naples Federico II (Italy) Luca Scalfi, Paola Vitaglione, and Concetta Montagnese. 11. Ghent University (Belgium) Ilse De Bourdeaudhuij, Stefaan De Henauw, Tineke De Vriendt, Lea Maes, Christophe Matthys, Carine Vereecken, Mieke de Maeyer, Charlene Ottevaere, and Inge Huybrechts. 12. Medical University of Vienna (Austria) Kurt Widhalm, Katharina Phillipp, Sabine Dietrich, Birgit Kubelka, and Marion Boriss-Riedl. 13. Harokopio University (Greece) Yannis Manios, Eva Grammatikaki, Zoi Bouloubasi, Tina Louisa Cook, Sofia Eleutheriou, Orsalia Consta, George Moschonis, Ioanna Katsaroli, George Kraniou, Stalo Papoutsou, Despoina Keke, Ioanna Petraki, Elena Bellou, Sofia Tanagra, Kostalenia Kallianoti, Dionysia Argyropoulou, Katerina Kondaki, Stamatoula Tsikrika, and Christos Karaiskos. 14. Institut Pasteur de Lille (France) Jean Dallongeville and Aline Meirhaeghe. 15. Karolinska Institutet (Sweden) Michael Sjöstrom, Jonatan R Ruiz, Francisco B. Ortega, María Hagströmer, Anita Hurtig Wennlöf, Lena Hallström, Emma Patterson, Lydia Kwak, Julia Wärnberg, and Nico Rizzo. 16. Asociación de Investigación de la Industria Agroalimentaria (Spain) Jackie Sánchez-Molero, Sara Castelló, Elena Picó, Maite Navarro, Blanca Viadel, José Enrique Carreres, Gema Merino, Rosa Sanjuán, María Lorente, and María José Sánchez. 17. Campden BRI (UK) Chantal Gilbert, Sarah Thomas, Elaine Allchurch, and Peter Burgess. 18. SIK - Institutet foer Livsmedel och Bioteknik (Sweden) Gunnar Hall, Annika Astrom, Anna Sverkén, and Agneta Broberg. 19. Meurice Recherche and Development asbl (Belgium) Annick Masson, Claire Lehoux, Pascal Brabant, Philippe Pate, and Laurence Fontaine. 20. Campden and Chorleywood Food Development Institute (Hungary) Andras Sebok, Tunde Kuti, and Adrienn Hegyi. 21. Productos Aditivos SA (Spain) Cristina Maldonado and Ana Llorente. 22. Cárnicas Serrano SL (Spain) Emilio García. 23. Cederroth International AB (Sweden) Holger von Fircks, Marianne Lilja Hallberg, and Maria Messerer 24. Lantmännen Food R&D (Sweden) Mats Larsson, Helena Fredriksson, Viola Adamsson, and Ingmar Börjesson. 25. European Food Information Council (Belgium) Laura Fernández, Laura Smillie, and Josephine Wills. 26. Universidad Politécnica de Madrid (Spain) Marcela González-Gross, Jara Valtueña, Ulrike Albers, Raquel Pedrero, Agustín Meléndez, Pedro J. Benito, Juan José Gómez Lorente, David Cañada, David Jiménez-Pavón, Alejandro Urzanqui, Francisco Fuentes, Rosa María Torres, and Paloma Navarro.

TABLE 1 Characteristics of the HELENA sample, as a function of the *FADS1* rs174546 genotypes[1]

	FADS1 rs174546 genotypes					
	CC	CT	TT	CT + TT	P^2	P^3
	n = 271	*n* = 237	*n* = 65	*n* = 302		
Boys/girls, *n*	134/137	121/116	26/39	147/155		
Age, *y*	14.8 ± 0.2	14.8 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	0.52	0.77
BMI, *kg/m²*	20.7 ± 0.3	20.3 ± 0.3	20.4 ± 0.4	20.3 ± 0.3	0.17	0.12
Serum phospholipid (n-6) FA						
LA, *% total FA*	21.4 ± 0.2	22.3 ± 0.2	23.3 ± 0.3	22.5 ± 0.2	<0.0001	<0.0001
ARA, *% total FA*	9.9 ± 0.1	8.9 ± 0.1	7.8 ± 0.2	8.7 ± 0.1	<0.0001	<0.0001
Serum phospholipid (n-3) FA						
ALA, *% total FA*	0.14 ± 0.01	0.16 ± 0.01	0.20 ± 0.01	0.17 ± 0.01	<0.0001	0.0002
EPA, *% total FA*	0.58 ± 0.03	0.54 ± 0.03	0.47 ± 0.04	0.52 ± 0.03	0.0002	0.003
DHA, % total FA	3.10 ± 0.09	3.00 ± 0.09	2.97 ± 0.13	2.99 ± 0.09	0.14	0.15
Serum lipids and lipoproteins						
Total cholesterol, *mmol/L*	4.15 ± 0.05	4.11 ± 0.05	4.04 ± 0.09	4.09 ± 0.05	0.23	0.35
HDL-cholesterol, *mmol/L*	1.45 ± 0.02	1.46 ± 0.02	1.42 ± 0.03	1.45 ± 0.02	0.54	0.87
Non-HDL–cholesterol, *mmol/L*	2.70 ± 0.04	2.66 ± 0.04	2.61 ± 0.08	2.65 ± 0.04	0.3	0.36
LDL cholesterol, *mmol/L*	2.40 ± 0.04	2.39 ± 0.04	2.38 ± 0.08	2.39 ± 0.04	0.77	0.76
TG, *mmol/L*	0.76 ± 0.02	0.75 ± 0.02	0.79 ± 0.04	0.76 ± 0.02	0.80	0.90
Usual dietary intake						
LA, *g/d*	10.2 ± 0.3	10.3 ± 0.3	10.3 ± 0.5	10.3 ± 0.3	0.69	0.69
ALA, *g/d*	1.59 ± 0.06	1.54 ± 0.07	1.49 ± 0.09	1.53 ± 0.06	0.18	0.24

[1] Values are means ± SEM adjusted for age, sex, and BMI.
[2] *P*-value under the additive model.
[3] *P*-value under the dominant model.

between the rs174546 genotype and lipid or lipoprotein variables in either group.

Likewise, there was no interaction between dietary ALA intake and rs174546 SNP on serum phospholipid levels of ALA, EPA, and DHA (**Table 3**; **Supplemental Table 3**). In both the low- and high-ALA–intake groups, the rs174546 T allele was associated with higher concentrations of ALA. The rs174546 T allele was significantly associated with lower serum phospholipid EPA levels in the high-ALA–intake group and a similar trend was observed in the low-ALA–intake group. In contrast, no association between the *FADS1* rs174546 polymorphism and DHA concentrations was observed in either group.

There was an interaction between ALA intake and the rs174546 polymorphism on total ($P = 0.006$) and non-HDL–cholesterol concentrations ($P = 0.01$) under the dominant model (Table 3). The rs174546 T allele was associated with lower serum total cholesterol ($P = 0.01$) and non-HDL–cholesterol ($P = 0.02$) concentrations in the high-ALA–intake group only. This association was only partially explained by lower LDL-cholesterol concentrations ($P = 0.11$). Under the additive model, similar results were obtained, although *P*-values for interaction were at borderline significance (*P*-interaction = 0.06 and 0.08 for total and HDL-cholesterol, respectively) (Supplemental Table 3). In contrast and whatever the genetic model tested, there was no association between *FADS1* rs174546 and lipid or lipoprotein concentrations in the low-ALA–intake group.

The LA and ALA intakes were correlated ($r = 0.81$; $P < 0.0001$). Adjustment for LA intake did not alter the significance of the associations between *FADS1* polymorphism and total or non-HDL–cholesterol concentrations in the high-ALA–intake

TABLE 2 Serum concentrations of (n-6) PUFA, lipids, and lipoproteins according to dietary LA intake and *FADS1* rs174546 genotypes under the dominant model[1]

	LA intake ≤9.4 g/d			LA intake >9.4 g/d			
	CC	CT + TT	*P*	CC	CT + TT	*P*	*P*-interaction
n	140	150		131	152		
LA intake, *g/d*	7.4 (6.6; 8.3)	7.7 (6.5; 8.6)	–	12.1 (10.4; 13.9)	11.9 (10.5; 15.1)	–	–
Serum phospholipid LA, *% total FA*	21.1 ± 0.3	22.1 ± 0.3	0.0004	21.7 ± 0.2	22.9 ± 0.2	0.0002	0.72
Serum phospholipid ARA, *% total FA*	10.0 ± 0.2	8.7 ± 0.2	<0.0001	9.8 ± 0.1	8.6 ± 0.1	<0.0001	0.48
Serum total cholesterol, *mmol/L*	4.17 ± 0.06	4.17 ± 0.06	0.96	4.12 ± 0.07	4.00 ± 0.06	0.12	0.29
Serum HDL-cholesterol, *mmol/L*	1.45 ± 0.02	1.44 ± 0.02	0.80	1.45 ± 0.02	1.45 ± 0.02	0.95	0.91
Serum non-HDL–cholesterol, *mmol/L*	2.72 ± 0.06	2.73 ± 0.06	0.86	2.68 ± 0.06	2.56 ± 0.06	0.13	0.26
Serum LDL–cholesterol, *mmol/L*	2.43 ± 0.06	2.47 ± 0.06	0.53	2.35 ± 0.06	2.30 ± 0.06	0.27	0.28
Serum TG, *mmol/L*	0.76 ± 0.03	0.75 ± 0.03	0.83	0.76 ± 0.03	0.76 ± 0.03	0.94	0.97

[1] Values are median and the IQR (Q1; Q3) or means ± SEM, adjusted for age, sex, BMI, and total energy intake.

TABLE 3 Serum concentrations of (n-3) PUFA, lipids, and lipoproteins according to dietary ALA intake and *FADS1* rs174546 genotypes under the dominant model[1]

	ALA intake ≤1.4 g/d			ALA intake > 1.4 g/d			
	CC	CT + TT	*P*	CC	CT + TT	*P*	*P*-interaction
n	135	159		136	143		
ALA intake, *g/d*	1.1 (1.0; 1.3)	1.1 (1.0; 1.3)	—	1.8 (1.6; 2.2)	1.8 (1.6; 2.3)	—	—
Serum phospholipid ALA, *% total FA*	0.14 ± 0.01	0.17 ± 0.01	0.0008	0.15 ± 0.01	0.17 ± 0.01	0.05	0.29
Serum phospholipid EPA, *% total FA*	0.57 ± 0.04	0.53 ± 0.04	0.14	0.59 ± 0.03	0.51 ± 0.03	0.007	0.15
Serum phospholipid DHA, *% total FA*	3.09 ± 0.12	3.01 ± 0.11	0.58	3.02 ± 0.10	2.89 ± 0.10	0.17	0.38
Serum total cholesterol, *mmol/L*	4.10 ± 0.07	4.20 ± 0.06	0.23	4.18 ± 0.06	3.98 ± 0.06	0.01	0.006
Serum HDL-cholesterol, *mmol/L*	1.43 ± 0.02	1.45 ± 0.02	0.52	1.46 ± 0.02	1.44 ± 0.02	0.51	0.34
Serum non-HDL–cholesterol, *mmol/L*	2.67 ± 0.06	2.75 ± 0.06	0.31	2.71 ± 0.05	2.54 ± 0.05	0.02	0.01
Serum LDL–cholesterol, *mmol/L*	2.39 ± 0.06	2.48 ± 0.06	0.26	2.41 ± 0.06	2.29 ± 0.06	0.11	0.06
Serum TG, *mmol/L*	0.74 ± 0.03	0.78 ± 0.03	0.45	0.79 ± 0.03	0.74 ± 0.03	0.25	0.12

[1] Values are median and the IQR (Q1; Q3) or means ± SEM, adjusted for age, sex, BMI, and total energy intake.

group. Further adjustment for physical activity level did not substantially change the results (data not shown). Lastly, we examined the interaction with ALA intake as a continuous variable to avoid the arbitrary selection of cutoff points (i.e. the medians). In agreement with the previous data, the interaction between ALA intake as a continuous variable and the *FADS1* rs174546 polymorphism (under the dominant model) remained significant for total cholesterol ($P = 0.03$) and was borderline significant for non-HDL–cholesterol ($P = 0.07$).

Discussion

In this study, we report a significant interaction between dietary ALA intake and the *FADS1* rs174546 polymorphism on serum total and non-HDL–cholesterol concentrations in European adolescents. High ALA intakes were associated with lower concentrations of total and non-HDL–cholesterol in carriers of rs174546 minor allele only (**Fig. 1**). Hence, our results replicate, in an independent sample, the recently reported interaction between (n-3) PUFA intake and rs174546 on serum total and non-HDL–cholesterol concentrations in 3575 Dutch adults (16) and extend these observations to adolescents. Taken as a whole, our data suggest that ALA intake modulates the impact of *FADS1* genetic variants on serum lipid concentrations and that this occurs at a young age.

Tissue or serum levels of LA and ALA and of their biologically active LC-PUFA derivatives are influenced not only by diet but to a larger extent also by genetic variants in the *FADS1-FADS2* gene cluster. Numerous studies (including genome-wide association studies) consistently showed an association between polymorphisms in the *FADS1-FADS2* gene cluster and the FA composition of adipose tissue (2), erythrocyte membranes (3,4, 6–8), and plasma or serum phospholipids (2–9). Carriers of minor alleles of these SNPs (including rs174546) had higher levels of desaturation substrates (such as LA, eicosadienoic acid, and ALA) and lower levels of desaturation products (such as ARA, EPA, and docosapentaenoic acid). It appears from these studies that minor alleles of these SNPs lead to a reduced efficiency of the endogenous synthesis of LC-PUFA from their precursors (LA and ALA). This hypothesis is further supported by Gieger et al. (27) who showed significant associations between the *FADS1* rs174548 polymorphism (which is in high linkage disequilibrium with rs174546) and a number of plasma glycerophospholipid concentrations (expressed in absolute values, μmol/L), suggesting a reduced efficiency of the D5D reaction. In addition, the rs174546 polymorphism tags ($r^2 \geq 0.8$) several other *FADS* cluster SNPs that have been associated with plasma lipid and lipoprotein concentrations in adults (14). Its minor allele is located within a microRNA target site and is associated with lower *FADS1* mRNA levels in human liver (14), suggesting that the rs174546 SNP is either functional or in linkage disequilibrium with a functional SNP affecting D5D activity.

We showed that the *FADS1* rs174546 T allele was associated with both higher serum phospholipid ALA concentrations and lower total and non-HDL–cholesterol concentrations in adolescents reporting a high dietary ALA intake (median 1.8 g/d) but

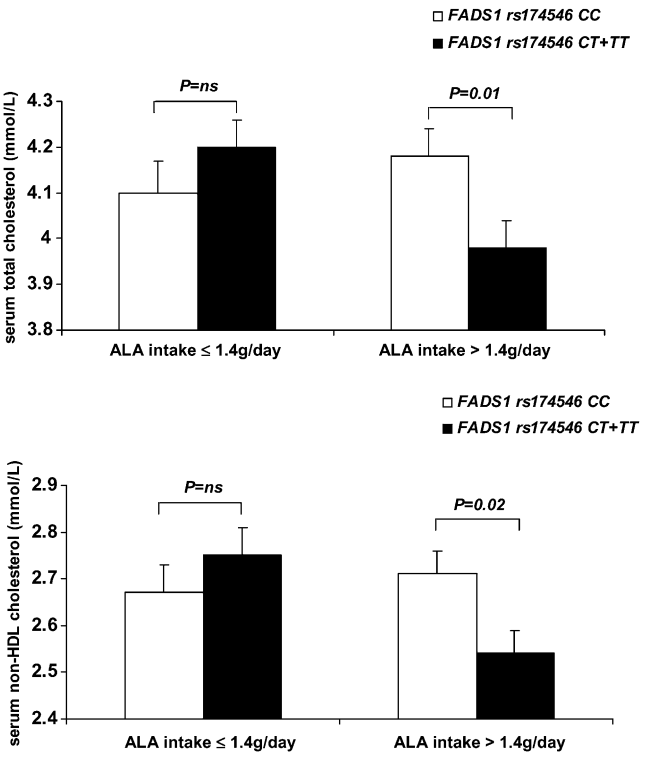

FIGURE 1 Association of the FADS1 rs174546 polymorphism with total and non-HDL–cholesterol in European adolescents by dietary ALA intake. Data are means ± SEM, $n = 135$–159 adjusted for age, sex, BMI, and total energy intake. ns, $P > 0.05$.

not in those with a low intake (median 1.1 g/d). The mechanisms underlying this association remain to be elucidated. However, this observation might explain the results of earlier trials assessing the effects of moderate ALA supplementation on blood lipid concentrations [for review, see (28)]. In these trials, moderate ALA supplementation (1.2–3.6 g/d) had no effect or inconsistent effects on plasma lipid and lipoprotein concentrations. In contrast, supplementation with high doses of ALA (8.8 g/d) lowers total and LDL-cholesterol concentrations (by 0.20 and 0.13 mmol/L, respectively) (29). In the present study, the combination of a modest ALA intake (median 1.8 g/d) and the raising effect of rs174546 T allele on ALA concentrations was sufficient to lower non-HDL–cholesterol concentrations.

The present study extends to adolescents the observation of an interaction between (n-3) PUFA intake and the *FADS1* rs174546 polymorphism on serum total and non-HDL–cholesterol that was recently reported in adults by Lu et al. (16). In contrast, the interaction between (n-6) PUFA intake and rs174546 on HDL-cholesterol concentrations (16) was not replicated here. The reasons for this apparent discrepancy are not clear. However, the significant changes in the plasma lipid/lipoprotein profile that occur during the transition from late childhood to adolescence (15) may have masked the interaction in our sample. We checked whether Tanner status modified this interaction and did not find a significant association (data not shown), suggesting that this is not a likely explanation. Alternatively, it cannot be excluded that dietary (n-3) and (n-6) PUFA intake differ between adults and adolescents. However, such differences are difficult to evaluate due to the lack of international standardization in both the dietary recall methods and the food composition tables used to determine the food content in PUFA. In addition, studies that compare the mean dietary intake of (n-3) and (n-6) PUFA between adults and adolescents are still scarce. Nevertheless, a comparison of the mean Australian intake of (n-3) and (n-6) PUFA between adults and children or adolescents aged 18 y and younger has shown that adults consume nearly twice as much LC-PUFA than children and adolescents, probably due to a higher consumption of fish and seafood products (30). In contrast, the mean intake of LA and ALA was relatively similar between adults (10.8 and 1.17 g/d, respectively) and adolescents aged from 12 to 15 y (10.7 and 1.22 g/d, respectively). Lastly, the discrepancy might be due to a lack of statistical power in the present study. With a minor allele frequency of 0.32, the statistical power to detect a significant association ($P < 0.05$) with HDL-cholesterol ($\beta = -0.019$ mmol/L) (14) in the high-LA–intake group was only 13%. Therefore, very large genetic association studies or meta-analyses are required to fully explore the interaction between dietary PUFA intake and *FADS1* polymorphism on lipid/lipoprotein metabolism in the young.

This study had several strengths and, conversely, a number of limitations. First, our analyses were restricted to the *FADS1* rs174546 polymorphism. However, as indicated above, this SNP shows the strongest associations with serum phospholipid levels of LC-PUFA in the HELENA study (9) and tags a cluster of SNPs associated with plasma lipid concentrations (14). Hence, this SNP was a logical choice for examining gene-diet interactions. Second, given that the serum phospholipid LC-PUFA concentrations were measured in the HELENA study, we were able to assess the relationship between dietary LA and ALA intakes and their corresponding serum phospholipid levels. The dietary intake of LA was weakly but significantly correlated with the serum phospholipid LA level as a percentage of total FA ($r = 0.15$; $P = 0.0004$), whereas no such correlation was found for ALA ($r = 0.03$; $P = 0.51$). This may possibly be explained by the lag (<1 wk) between the two 24-h recalls and the blood sample collection and by the fact that PUFA were measured in serum phospholipid fraction rather than in adipose tissue. However, although adipose tissue is a preferred medium for the measurement of FA as a reflection of long-term dietary intakes, it was shown that at least for several PUFA, including LA, PUFA concentrations in serum phospholipids may reflect intake of FA over the past few days or more (31–33). In addition, our results are consistent with those of other studies having compared PUFA intake with biomarker concentrations in adults (33,34). Third, the 24-h dietary recall method used in the present study has some limitations. The accuracy of collected data relies on the individual's ability to remember the composition of previous meals, including mixed dishes. Accordingly, there is a risk of misreporting. In addition, adolescents do not usually know which food preparation methods have been applied (e.g. the choice of cooking fat). However, use of the computer-assisted HELENA-DIAT tool standardized the 24-h dietary recalls as much as possible. Nutrient intakes were corrected for within- and between-person variability by applying the MSM method and average values for cooking fat use were estimated according to each country's usual preparation methods. Nevertheless, our results would benefit from replication with an independent sample of adolescents.

In conclusion, this study confirmed the gene-nutrient interaction between ALA intake and the *FADS1* rs174546 polymorphism on non-HDL–cholesterol concentrations recently shown in adults and has extended the findings to European adolescents. This interaction may help to explain the interindividual differences in plasma cholesterol concentrations observed in response to (n-3) PUFA dietary content. However, this point requires further investigation with appropriate interventional studies.

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F.G., K.W., A.K., D.M., M.G., L.M., and J. Dallongeville conceived and designed the study; A.S., E.G., N.B., K.V., K.W., A.K., D.M., I.L., and L.M. acquired the data; J. Dumont, I.H., P.A., A.M., and J. Dallongeville analyzed and interpreted the data; J. Dumont, I.H., A.M., and J. Dallongeville wrote the manuscript; A.S., F.G., E.G., K.V., I.L., M.G., and L.M. critically revised the manuscript; and J. Dumont, A.M., and J. Dallongeville had primary responsibility for the final content. All authors read and approved the final manuscript.

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