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Intake levels of dietary long-chain polyunsaturated fatty acids modify the association between genetic variation in *FADS* and LDL cholesterol

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Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; BMI, body mass index; BMR, basal metabolic rate; CVD, cardiovascular disease; DHA, docosahexanoic acid; DPA, docosapentanoic acid; E%, energy percent; EPA, eicosapentanoic acid; FADS, fatty acid desaturase; GLA, gamma-linolenic acid; LA, linoleic acid; MDC, Malmö Diet and Cancer cohort; MDC-CC, Malmö Diet and Cancer Cardiovascular Cohort; mRNA, messenger RNA; PAL, physical activity level; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acids; SNP, single nucleotide polymorphism.

ABSTRACT

Polymorphisms of the fatty acid desaturase gene cluster *(FADS)* have been associated with LDL, HDL and triglyceride concentrations. Because FADS converts α-linolenic acid and linoleic acid into long-chain polyunsaturated fatty acids (PUFA), we investigated the interaction between different PUFA intakes and the *FADS* polymorphism rs174547 (T>C) on fasting blood lipid and lipoprotein concentrations. We included 4,635 individuals (60% females, 45-68 years) from the Swedish population-based Malmö Diet and Cancer cohort. Dietary intakes were assessed by a modified diet history method including 7-day registration of cooked meals. The C-allele of rs174547 was associated with lower LDL concentration (P=0.03). We observed significant interaction between rs174547 and long-chain ω-3 PUFAs intakes on LDL (P=0.01); the C-allele was only associated with lower LDL among individuals in the lowest tertile of long-chain ω-3 PUFA intakes (P<0.001). In addition, significant interaction was observed between rs174547 and the ratio of α-linolenic and linoleic fatty acid intakes on HDL (P=0.03). However, no significant associations between the C-allele and HDL were detected within the intake tertiles of the ratio. Our findings suggest that dietary intake levels of different PUFAs modify the associated effect of genetic variation in *FADS* on LDL and HDL.

Supplementary key words; diet, fatty acid desaturase, polyunsaturated fatty acids, cholesterol, cohort, epidemiology

INTRODUCTION

The blood concentrations of LDL cholesterol, HDL cholesterol and triglycerides have a strong genetic influence (1, 2). In recent genome-wide association studies, single nucleotide polymorphisms (SNPs) in the fatty acid desaturase gene cluster *(FADS)* that includes *FADS1, FADS2* and *FADS3* genes, were associated with LDL-, HDL-, triglyceride- (3-5) and fasting glucose concentrations (6-8). First, an association was observed between the C-allele of rs174547 in *FADS1* and decreased HDL and increased triglyceride concentrations in a study comprising almost 40,000 Europeans (4). The same allele was associated with lower mRNA levels of *FADS1* and *FADS3* in liver (4). More recently, a meta-analysis of >100,000 individuals reported genome-wide significant associations of the *FADS* locus and LDL-, HDL- as well as triglyceride concentrations (5).

FADSs are key-enzymes in the endogenous desaturation of α-linolenic acid (ALA, C18:3ω-3) and linoleic acid (LA, C18:2ω-6) into long-chain polyunsaturated fatty acids (PUFAs), where FADS1 is a Δ-5 desaturase and FADS2 a Δ-6 desaturase (9, 10). Further, SNPs in the *FADS* locus have been associated with blood concentrations of long-chain PUFAs as well as with cholesterol concentrations (9, 11-16). Long-chain PUFAs regulate the fluidity of cell membrane, act as second messengers in intracellular signaling pathways and regulate transcription (17). Dietary intakes of the long-chain ω-3 PUFAs, docosahexanoic acid (DHA, C22:6ω-3) and eicosapentanoic acid (EPA, C20:5ω-3), have been reported to lower serum triglyceride levels (18), and higher dietary intake of ω-3

was associated with higher HDL and LDL in the Malmö Diet and Cancer (MDC) cohort (19). Additionally, long-chain PUFAs, such as arachidonic acid (AA, C20:4ω-6) and EPA, are precursors for inflammatory molecules such as eicosanoids (9, 20) and high long-chain PUFA concentration has been associated with lower prevalence of both metabolic syndrome and cardiovascular disease (CVD) (9, 10). Further, previous studies have suggested that dietary intake levels of different PUFAs interact with *FADS1* variation to affect blood lipids (13, 21).

Because FADS are key regulators of ALA and LA desaturation, we examined if different dietary intake levels of PUFAs modify the association between the rs174547 polymorphism in the *FADS* locus and blood concentrations of LDL, HDL and triglycerides.

SUBJECTS AND METHODS

Study population

The MDC cohort is a population-based prospective cohort including 28,449 participants, with baseline data collection conducted throughout the years 1991-96 (22). The study population include individuals born during 1923-50 (23) and living in Malmö, the third largest city in Sweden with about 295,000 citizens. The participation rate was approximately 40% (24). Among MDC participants recruited from November 1991 to February 1994 (n=12,445), a random 50% was invited to further participate in a carotid artery disease study, the Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC). In total, 6,103 individuals underwent a review of their medical history, a physical

examination and a laboratory assessment of cardiovascular risk factors (22, 24, 25). Information on LDL, HDL and triglycerides fasting blood concentrations was available in 5,363 individuals. Totally, information on diet, *FADS* genotype and blood lipids were available in 4,943 individuals. After excluding individuals with diabetes mellitus (self-reported diagnosis or using anti-diabetes medication, n=123), users of lipid-lowering medication (n=117) and those with a history of cardiovascular event (coronary event or stroke, n=117) the study sample included 4,635 individuals (45-68 years, 60.3% females). Information about the history of cardiovascular event (coronary event or stroke) was taken from the national Swedish Hospital Discharge register and the local register of stroke (24). All individuals provided a written informed consent and the ethics committee of Lund University approved the MDC study protocols.

Genotyping

The genotyping of the *FADS* rs174547 (T/C) was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry on the Sequenom Mass-ARRAY platform (San Diego, CA). Genotyping was successful in 5,806 (96%) of the 6,055 individuals with DNA available and the rs174547 was in Hardy-Weinberg equilibrium (P=0.92). In addition, 5,490 of the 6,055 individuals, of whom we still have DNA available, were additionally genotyped by the TaqMan allelic discrimination on ABI 7900 with a concordance rate for the two methods of 99.2%.

Dietary information

Dietary intake was measured by a modified diet history methodology combining a 168-item dietary questionnaire, a 7-day menu book and a 1-h diet history interview, especially designed for the MDC study (26). The 168-item dietary questionnaire covered food items regularly consumed during the past year. The participants were asked to fill in the frequency of food intake and estimate the usual portion sizes using a booklet with photographic aids. The 7-day menu book covered cooked lunch and dinner meals, cold beverages (including alcoholic beverages), medications, natural remedies and dietary supplements used by the participants. To complete the dietary data the participants were interviewed, for one hour, about their food choices, food preparation practices and portion sizes (by using a more extensive booklet of photos) of the food reported in the menu book. The trained interviewers checked the menu book and questionnaire for very high reported intakes and overlapping information. The average daily food intake (grams per day) was calculated based on the information from the menu book, interview and questionnaire, and converted into nutrient and energy intakes by using the MDC Food and Nutrient Database, developed from the PC KOST-93 of the Swedish National Food Administration (26).

The different PUFA intakes were converted into the percentage contributed by the specific PUFAs to total energy intake (E%). The PUFAs dietary variables included in this study were: ALA (C18:3ω-3); long-chain ω-3 PUFA (EPA [C20:5ω-3], docosapentanoic acid [DPA, C22:5ω-3] and DHA [C22:6ω-3]); total ω-3 PUFA (ALA, EPA, DPA and DHA); total ω-6 PUFA (LA [C18:2ω-6], γ-linolenic acid [GLA, C18:3ω-6] and AA

[C20:4ω-6]); ALA to LA ratio (ALA/LA); total ω-3 to total ω-6 PUFA ratio (ω-3/ω-6 PUFA).

The relative validity of the modified diet history method was examined in 105 women and 101 men. As the reference method a total of 18 days of weighed food records was collected during 3 consecutive days, every second month during one year. The energy-adjusted correlation coefficients between the modified diet history method and the reference method were in men: 0.22 for ALA, 0.23 for LA, 0.55 for AA, 0.24 for EPA, 0.37 for DPA and 0.20 for DHA. In women, the coefficients were 0.58 for ALA, 0.68 for LA, 0.44 for AA, 0.38 for EPA, 0.40 DPA and 0.27 for DHA (27).

Other variables

HDL, triglyceride and total cholesterol concentrations were determined in over-night fasting blood samples by standard methods at the department of Clinical Chemistry, University Hospital of Malmö. LDL concentration was calculated by the Friedewald formula: LDL = total cholesterol – HDL – (triglycerides/2.2) (24). To individuals with a triglyceride concentration of more than 400 mg per deciliter (4.5 mmol per liter), LDL was defined as missing (25). Body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters (kg/m^2).

A self-administered questionnaire was used to determine lifestyle factors including cigarette smoking, alcohol intake, and physical activity habits. Three categories of smoking status were used: current (including irregular smoking), former and never

smokers. Alcohol habits were divided into five categories. Individuals reporting no alcohol consumption during the last year in the questionnaire, which also were zero reporters of alcohol in the 7-day menu book, were categorized as zero-consumers of alcohol. We divided the other study participants' alcohol consumption (grams per day) into categories with different cut-offs according to gender. The cut-off levels for females were 5, 10, and 20 grams of alcohol per day and the cut-off levels for males were 10, 20 and 40 grams of alcohol per day. The leisure time physical activity level was calculated from a list of 17 different activities in the questionnaire. The time spent on each activity was multiplied with an intensity factor, creating a leisure time physical activity score. Leisure time physical activity score was then divided into quintiles, with the same cut-offs for both genders. Separate categories for smoking, alcohol intake and leisure time physical activity were constructed for the subjects with missing data.

Statistical analysis

SPSS Inc. PASW Statistics 18.0 was used for statistical analysis. Statistical significance was set at $P<0.05$ and all *P*-values are 2-sided. All of the covariates, except season, were differently distributed between tertiles of total ω-3 and ω-6 PUFA intake. Assuming an additive model, associations with the *FADS* rs174547 (T/C) genotype categories were investigated by using General Linear Model adjusted for age and sex (basic analysis) and thereafter age, sex and BMI. Interaction between *FADS* genotype and tertiles of dietary intake levels on serum lipid concentrations were studied by introducing a multiplicative factor of genotypes and diet tertiles as continuous variables in addition to these main factors as separate variables. The interaction analyses were adjusted for potential

confounders: age, sex, BMI, season of diet collection (4 categories), cigarette smoking, leisure time physical activity, alcohol intake, and total energy intake. All continuous variables except age were Ln transformed to achieve normal distribution when testing for trend across *FADS* genotype categories and interaction between *FADS* genotype categories and tertiles of dietary intake levels of PUFA on LDL, HDL and triglycerides; before transformation, a very small amount (0.001 g) was added to ω-3 PUFA intake to handle zero intakes.

In sensitivity analyses, potential misreporters of energy were excluded. Misreporters of energy intake were identified by comparing the individually estimated physical activity level (PAL) expressed as the energy expenditure divided by the basal metabolic rate (BMR) with energy intake divided by BMR, further explained elsewhere (28). Individuals were defined as misreporters when the ratio of the reported energy intake to BMR was outside the 95% confidence limits of the calculated PAL (i.e. under- and over-reporters).

RESULTS

Each C-allele of rs174547 associated with 0.05 mmol/L lower LDL concentration (*P*-trend=0.03, **Table 1**), but not with HDL or triglyceride concentrations (*P*-trend=1.00 and *P*-trend=0.10, respectively) in the basic analysis. BMI was significantly associated with genotypes and when BMI was included as a covariate, a significant association of 0.02 mmol/L higher triglyceride concentration per C-allele was observed (*P*-trend=0.04).

Similar to the basic analysis, the association with LDL concentration remained significant (*P*-trend=0.047), and no association with HDL concentration was detected (*P*-trend=0.52) after adjusting for BMI.

PUFA intakes did not differ according to *FADS* genotypes (**Table 1**). No significant interaction was observed between the *FADS* genotype categories and intake levels of total ω-3 PUFAs on LDL concentration (**Table 2**). However, we observed a significant interaction between the *FADS* genotype categories and long-chain ω-3 PUFA intake on LDL concentration (*P*=0.01). The C-allele was significantly associated with lower LDL among individuals within the lowest tertile of long-chain ω-3 PUFA intake (≤0.14 E%, *P*<0.001), but not among those in the mid- (0.14-0.28 E%) or highest tertiles (>0.28 E%). When examining within genotype categories, the high long-chain ω-3 PUFA intake associated significantly with higher LDL concentration among the CC-genotype- (*P*<0.001) and TC-genotype carriers (*P*=0.04) but not among TT-genotype carriers (*P*=0.17) (**Fig. 1**). In addition, there was a significant interaction between the *FADS* genotype categories and ALA/LA intakes on HDL concentration (*P*=0.03) despite lack of significant associations between the *FADS* genotypes and HDL concentration in any of the ALA/LA tertiles. However, we observed significant associations between ALA/LA and HDL concentration among CC- (*P*=0.046) and TC-genotype carriers (*P*=0.02) but not among those with the TT-genotype (**Fig. 2**). No significant interactions were observed between the *FADS* genotype categories and any of the different PUFA intake levels on triglyceride concentration (**Table 2**).

We also examined interactions separately for men and women. We observed a significant interaction of the *FADS* genotype categories and long-chain ω-3 PUFA on LDL concentrations in men (P=0.01), but not in women (P=0.39). However, these interactions did not significantly differ between the genders (P=0.08) and there was no significant difference in gender distribution across the tertiles of long-chain ω-3 PUFA. No significant interactions of *FADS* genotype categories and ALA/LA on the levels of HDL were detected, or any of the different PUFA intakes on triglycerides levels, neither in men nor women.

There were significantly more males than females in the highest tertiles of total n-3 and n-6 PUFA intake compared to the mid- and lowest tertiles (P<0.001). Because the gender distribution across the tertiles of ω-3 and ω-6 PUFA intakes was not equal we repeated all the interaction analyses using gender specific cut-offs for the long-chain ω-3 and ω-6 PUFA tertiles. However, the above reported results remained essentially the same when using gender-specific cut-offs.

In sensitivity analyses, when excluding suspected misreporters of energy (19.3% of the study sample), the interaction between the *FADS* genotype categories and long-chain ω-3 PUFA intakes on LDL remained significant (P=0.04). The interaction between the *FADS* genotype categories and ALA/LA intake levels on HDL cholesterol was slightly attenuated after excluding misreporters (P=0.06). Interactions between *FADS* genotype categories and the different PUFA intake levels on triglyceride remained non-significant.

DISCUSSION

This study indicates that the dietary intake levels of long-chain ω-3 PUFAs may modify the association between *FADS* rs174547 and LDL concentration. Additionally, the intake levels of ALA/LA may modify the associated effect of *FADS* genotype categories on HDL concentrations.

Although several earlier studies, similar to our results, have found an association between the minor allele of rs174547 (or a SNP in high allelic association) and decreased LDL concentrations (6, 13, 29-31) some inconsistency between the reported associations with lipids and lipoproteins remains. Kathiresan et al. demonstrated that the minor C-allele of rs174547 associated with lower HDL and higher triglyceride concentrations in Caucasians (4) and similar associations were observed in a Japanese population (21). However, as the same C-allele was found to associate significantly with decreased LDL, but not with HDL or triglycerides in a Mongolian population, it was suggested that differences in dietary fish intake between Japanese and Mongolians could provide an explanation of the dissimilar results (21). The Mongolian diet is mainly based on livestock products with a very low intake of fish compared to the Japanese diet which in general includes a very large amount of fish products. Consistently, Japanese individuals had higher plasma concentrations of ω-3 PUFA and a higher ω-3/ω-6 PUFA compared to Mongolians (32). In our study, the rs174547 C-allele was associated with lower LDL concentration only in the lowest intake tertile of long-chain ω-3 PUFA. As the dietary source of long-chain fatty acids mainly comes from fatty fish, our finding is in agreement

with Nakayama et al.'s suggestion that among individuals with low fish intake, in our study represented by those in the lowest intake tertile of long-chain PUFAs, the association between the *FADS* genotype categories and LDL concentrations may be accentuated.

In contrast to Lu et al. who reported a significant association between the minor allele of rs174546 (corresponding to the C-allele of rs174547 of our study) and lower HDL concentration among individuals with high consumption of ω-6 PUFAs (≥5.26 E%) (13), we observed no significant interaction between ω-6 PUFA intakes and the *FADS* genotype on HDL concentration. However, as low intake of ω-6 PUFA could indirectly reflect a high ALA/LA intake, our observation of interaction between the *FADS* genotype categories and ALA/LA intakes on HDL cholesterol concentration, as well as association between high ALA/LA intake and higher HDL concentration among the C-allele carriers, could be in line with findings by Lu et al.

FADS1 is responsible for the desaturation of ALA and LA into EPA and AA (9, 10). Kathiresan et al. demonstrated that the minor allele of rs174547 (in our study associated with lower LDL concentration) was associated with lower gene expression of the *FADS1* and *FADS3* transcripts. Tanaka et al. showed that individual's homozygote for the minor allele of a SNP in high allelic association with *FADS* rs174547, had lower plasma concentrations of AA compared to individuals homozygote for the major allele. The SNP accounted for 18.6% of the additive variance in AA concentration (31). The potential mechanism for association between high ALA/LA intake and higher HDL among the

minor allele carriers could be that the minor allele, which has been associated with a lower desaturase activity (14), may affect the availability of long-chain ω-3 PUFAs differently between the genotypes. The metabolic derivatives, eicosanoids, of these long-chain ω-3 PUFAs are potent activators of peroxisome proliferator-activated receptors (PPAR) (9, 20) which can regulate the expression of genes directly involved in HDL production (13). Therefore, our result may reflect a limited long-chain PUFA availability among rs174547 minor allele carriers and thereby association between HDL concentrations and ALA/LA intake.

The strengths of our study include a relatively large sample size and detailed information on dietary intakes based on a 168-item dietary questionnaire, a 7-day menu book and a 1-h interview. However, the estimated dietary intakes were based on self-reports, and the limitation of a short term diet measurement to reflect the "habitual" intake may have introduced misclassification of dietary intakes and attenuation of the associations. Although dietary data from the MDC is in general of very high relative validity (33), the relative validity of some PUFAs still is rather low, especially long-chain ω-3 PUFAs in men, which is a weakness of the current study. One reason for the low validity may be the infrequent consumption of fatty fish (major source of long-chain ω-3 PUFAs) among many of the study participants. As the fish consumed at main meals was only registered during a limited number of 7 days, misclassification may be a problem. Furthermore, this is a cross-sectional study which limits our possibility to investigate causality. Finally, we performed multiple tests and thus some of the observed significant associations and interactions could be due to chance and need to be replicated.

It is generally accepted that genetic and environmental factors influence the blood lipid and lipoprotein concentrations but very little is known about such interactions. Our findings suggest that the dietary intakes of different PUFAs may modify the associated effect of the genetic variation in *FADS* on LDL and HDL concentration. Our results emphasize the importance of dietary fat composition in modifying the effect of genetic susceptibility on blood lipid and lipoprotein concentrations and highlight the potential of developing individualised prevention strategies of dyslipidemia and cardiovascular disease in the future. However, for such strategies to become possible, a major challenge now is to learn more about the importance and mechanisms of interactions between genetic variants, dietary intakes and blood lipid concentrations. It is therefore important to replicate our results in well-powered studies with good quality dietary data.

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FIGURE LEGENDS

Fig. 1. Association between rs174547 and LDL in strata of long-chain ω-3 PUFA among 4,635 individuals. The CC-genotype was associated with 0.14 mmol/L lower LDL concentration among individuals with low intakes of long-chain ω-3 PUFA (≤0.14 E%, $P=2.3x10^{-4}$), but not among those with medium (0.14-0.28 E%) or high (>0.28 E%) intakes ($P=0.98$ and $P=0.86$ respectively). A low intake of long-chain ω-3 PUFA was associated with 0.20 mmol/L and 0.06 mmol/L higher HDL concentration among individuals with CC-genotype ($P=2.7x10^{-3}$) and TC-genotype ($P=0.04$) but not among those with TT genotype ($P=0.17$). E%, energy percentage of total energy intake; PUFA, polyunsaturated fatty acid.

Fig. 2. Association between ALA/LA and HDL in strata of rs174547 among 4,635 individuals. A high ALA/LA was associated with 0.04 mmol/L and 0.02 mmol/L higher HDL concentration among individuals with CC-genotype ($P=0.046$) and TC-genotype ($P=0.02$) but not among those with TT genotype ($P=0.96$). There was no association between the CC-genotype and HDL in individuals with low, medium or high ALA/LA ($P=0.07$, $P=0.35$ and $P=0.35$ respectively). ALA, α-linolenic acid; E%, energy percentage of total energy intake; LA, linoleic acid.

TABLE 1. Characteristics of the Malmö Diet and Cancer –cardiovascular cohort individuals by *FADS1* rs174547 genotype.

Characteristics	**All (N=4,635)**	**T/T (N=2,054)**	**T/C (N=2,056)**	**C/C (N=525)**	***P*-trend[a]**
Women, N (%)	2795 (60.3)	1227 (59.7)	1240 (60.3)	328 (62.5)	0.52
Age (yr)	57.7 (52.3-62.6)	57.6 (52.2-62.3)	57.7 (52.2-62.6)	58.0 (52.2-62.7)	0.54
BMI (kg/m^2)	25.1 (22.8-27.7)	25.3 (23.0-27.8)	25.0 (22.9-27.3)	24.8 (22.7-27.3)	0.047
Fasting glucose (mmol/L)	4.9 (4.6-5.3)	4.9 (4.6-5.3)	4.9 (4.6-5.3)	4.9 (4.6-5.2)	0.44
LDL cholesterol (mmol/L)	4.10 (3.5-4.8)	4.10 (3.5-4.8)	4.10 (3.5-4.8)	4.00 (3.4-4.7)	0.03
HDL cholesterol (mmol/L)	1.35 (1.1-1.6)	1.36 (1.1-1.6)	1.36 (1.1-1.6)	1.36 (1.1-1.6)	1.00
Triglycerides (mmol/L)	1.14 (0.9-1.6)	1.13 (0.9-1.5)	1.13 (0.9-1.6)	1.18 (0.9-1.6)	0.10
Dietary intake					
ALA (E%)	0.73 (0.6-0.9)	0.73 (0.6-0.9)	0.72 (0.6-0.9)	0.71 (0.6-0.8)	0.06
Long-chain ω-3 PUFA (E%)	0.20 (0.1-0.3)	0.20 (0.1-0.3)	0.20 (0.1-0.3)	0.21 (0.1-0.4)	0.41
Total ω-3 PUFA (E%)	0.97 (0.8-1.2)	0.98 (0.8-1.2)	0.97 (0.8-1.2)	0.96 (0.8-1.2)	0.47
Total ω-6 PUFA (E%)	4.89 (4.0-5.9)	4.93 (4.1-5.9)	4.87 (4.0-5.9)	4.83 (4.0-5.7)	0.27

ALA/LA (E%)	0.15 (0.1-0.2)	0.15 (0.1-0.2)	0.15 (0.1-0.2)	0.15 (0.1-0.2)	0.44
Total ω-3/ω-6 PUFA (E%)	0.19 (0.2-0.2)	0.19 (0.2-0.2)	0.19 (0.2-0.2)	0.19 (0.2-0.2)	0.72

Data is median (inter-quartile range) or number (%), if not otherwise indicated. [a]Ln transformed. Adjusted for age and sex.

ALA, alpha-linolenic acid; BMI, body mass index; E%, energy percentage of total energy intake; LA, linoleic acid; PUFA, polyunsaturated fatty acids. Sex, age, diet variables and LDL, HDL and triglycerides (n=4,635). BMI (n=4,633).

Fasting glucose (n=4,624).

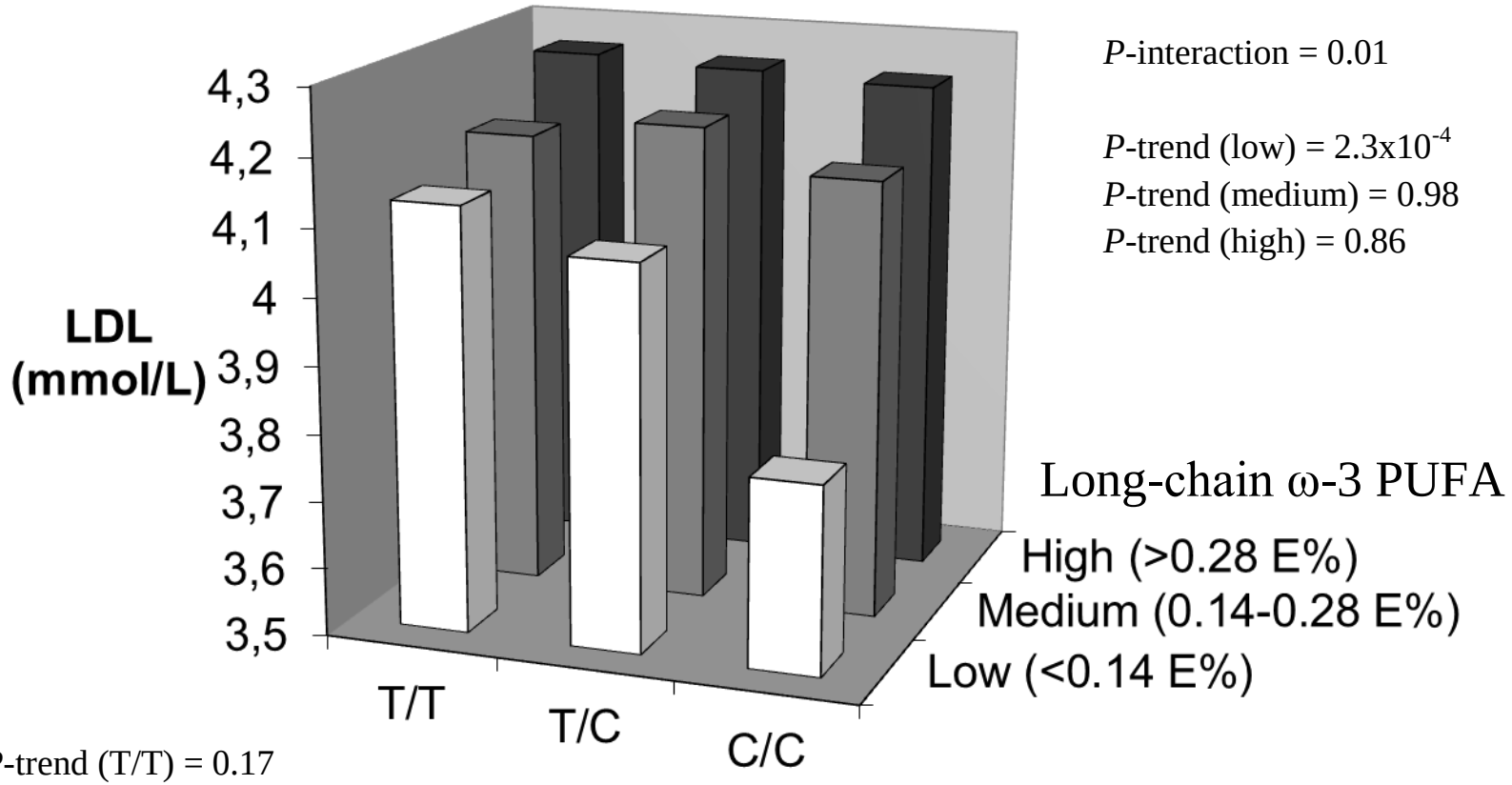
TABLE 2. Association between rs174547 (T/C) for each additional C-allele and blood lipids in strata of diet intakes among 4,635 individuals.

	LDL cholesterol			**HDL cholesterol**			**Triglycerides**		
Diet variables (E%)	Effect size	*P*-trend[a]	*P*-int[b]	Effect size	*P*-trend[a]	*P*-int[b]	Effect size	*P*-trend[a]	*P*-int[b]
ALA			0.94			0.55			0.47
Low (≤0.65)	-0.034	0.39		-0.003	0.81		0.024	0.37	
Medium (0.65-0.80)	-0.078	0.03		0.003	0.98		0.028	0.29	
High (≥0.80)	-0.021	0.55		0.009	0.71		0.013	0.89	
Long-chain ω-3 PUFA			0.01			0.53			0.70
Low (≤0.14)	-0.138	<0.001		-0.006	0.43		0.025	0.30	
Medium (0.14-0.28)	0.001	0.98		0.007	0.69		0.020	0.53	
High (≥0.28)	-0.007	0.86		0.004	0.95		0.025	0.49	
Total ω-3 PUFA			0.38			0.78			0.87

Low (≤0.86)	-0.071	0.05		0.004	0.67		0.006	0.91	
Medium (0.86-1.09)	-0.049	0.24		0.005	0.93		0.037	0.17	
High (≥1.09)	-0.013	0.62		0.001	0.78		0.024	0.55	
Total ω-6 PUFA			0.35			0.90			0.90
Low (≤4.35)	-0.052	0.16		0.002	0.89		0.013	0.53	
Medium (4.35-5.48)	-0.074	0.04		0.004	0.97		0.025	0.33	
High (≥5.48)	-0.007	0.83		0.002	0.86		0.030	0.53	
ALA/LA			0.49			0.03			0.15
Low (≤0.14)	-0.022	0.51		-0.020	0.07		0.041	0.17	
Medium (0.14-0.16)	-0.055	0.12		0.015	0.35		0.040	0.25	
High (≥0.16)	-0.059	0.12		0.014	0.35		-0.013	0.78	
Total ω-3/ω-6 PUFA			0.73			0.26			0.16
Low (≤0.17)	-0.056	0.11		-0.002	0.58		0.035	0.30	
Medium (0.17-0.22)	-0.018	0.77		-0.007	0.67		0.043	0.10	
High (≥0.22)	-0.064	0.05		0.015	0.38		-0.007	0.64	

Effect size (β) = difference in lipid concentration for each additional C-allele. [a]Adjusted for age and sex; [b]Adjusted for age, sex, season, alcohol intake, cigarette smoking, leisure time physical activity, BMI and total energy intake. ALA, α-linolenic acid; BMI, body mass index; E%, energy percentage of total energy intake; LA, linoleic acid; PUFA, polyunsaturated fatty acids.

Fig. 1

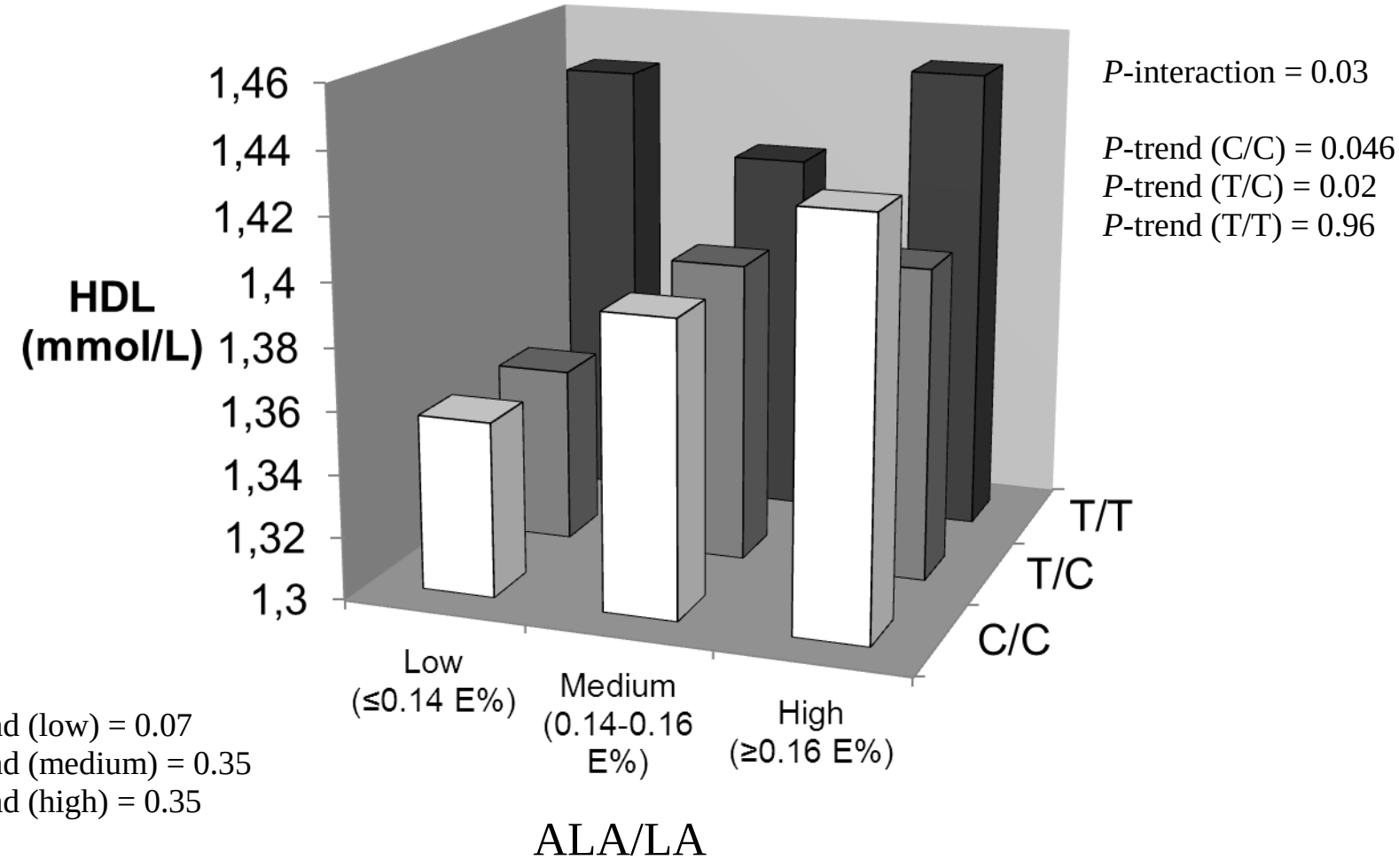


P-interaction = 0.01

P-trend (low) = 2.3×10^{-4}
P-trend (medium) = 0.98
P-trend (high) = 0.86

P-trend (T/T) = 0.17
P-trend (T/C) = 0.04
P-trend (C/C) = 2.7×10^{-3}

Fig. 2



P-interaction = 0.03

P-trend (C/C) = 0.046
P-trend (T/C) = 0.02
P-trend (T/T) = 0.96

P-trend (low) = 0.07
P-trend (medium) = 0.35
P-trend (high) = 0.35