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Does polymorphisms in *PPAR* and *APOE* genes modify associations between fatty acid desaturase (*FADS*), *n*-3 long-chain PUFA and cardiometabolic markers in 8–11-year-old Danish children?

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Abstract

n-3 Long-chain PUFA (LCPUFA) can improve cardiometabolic blood markers, but studies in children are limited. SNP in the *FADS* genes, which encode fatty acid desaturases, influence endogenous LCPUFA production. Moreover, SNP in genes that encode PPAR and apoE may modulate the effects of *n*-3 LCPUFA. We explored whether *FADS* polymorphisms were associated with blood cholesterol and TAG, insulin and glucose and whether polymorphisms in *PPAR* and *APOE* modified associations between *FADS* or *n*-3 LCPUFA status and the cardiometabolic blood markers. We measured fasting cholesterol and TAG, insulin, glucose and *n*-3 LCPUFA in 757 Danish 8–11-year-old children and genotyped SNP in *FADS* (rs1535 and rs174448), *PPARG2* (rs1801282), *PPARA* (rs1800206) and *APOE* (rs7412+rs429358). Carriage of two *FADS* rs174448 major alleles was associated with lower TAG ($P = 0{\cdot}027$) and higher HDL-cholesterol ($P = 0{\cdot}047$). Blood *n*-3 LCPUFA was inversely associated with TAG and insulin in *PPARG2* minor allele carriers and positively with LDL-cholesterol in major allele homozygotes ($P_{n\text{-}3\ \mathrm{LCPUFA} \times \mathrm{rs180182}} < 0{\cdot}01$). Associations between *n*-3 LCPUFA and cardiometabolic markers were not modified by *APOE* genotype ($P_{n\text{-}3\ \mathrm{LCPUFA} \times \mathit{APOE}} > 0{\cdot}11$), but interaction between *FADS* rs1535 and *APOE* showed that rs1535 major allele homozygotes who also carried *APOE2* had higher HDL-cholesterol than all other genotype combinations ($P_{\mathrm{rs1535} \times \mathit{APOE}} = 0{\cdot}019$, pairwise-$P < 0{\cdot}05$). This indicates that *FADS* genotypes, which increase endogenous LCPUFA production, may beneficially affect children's cardiometabolic profile in a partly *APOE*-dependent manner. Also, the degree to which children benefit from higher *n*-3 LCPUFA intake may depend on their *PPARG2* genotype.

Key words: **TAG: Cholesterol: Insulin: Glucose: Genotypes: EPA: DHA**

Dietary intake of *n*-3 long-chain PUFA (LCPUFA) modulates the plasma lipid profile in adults mainly by lowering circulating TAG, and sometimes also increasing LDL- and HDL-cholesterol[1]. We have also demonstrated this in a few studies in children[2,3]. However, results from intervention studies vary, and there is limited knowledge about the impact of genotype on the response to *n*-3 LCPUFA[4], especially in children. Such insight may help understand the mechanisms of action of *n*-3 LCPUFA and identify persons who may benefit the most from an increased consumption.

Fish is the main source of the *n*-3 LCPUFA, EPA and DHA, but *n*-3 LCPUFA are also endogenously produced from α-linolenic acid by the action of the fatty acid desaturase (FADS) enzymes. SNP in the *FADS1* and *FADS2* genes, which encode the D5- and D6-desaturase, respectively, have been shown to reduce enzyme activities and endogenous LCPUFA production and status[5–8]. The rs1535 SNP in the intronic region of *FADS2* and rs174448 between *FADS2* and *FADS3*, which tags SNP in *FADS2* genes, has both been associated with *n*-3 LCPUFA status in Danish infants[6]. Thus, *FADS* SNP may help explore the role of *n*-3 LCPUFA status on health outcomes. Carriage of *FADS* minor alleles has been linked to higher plasma TAG and lower HDL- and LDL-cholesterol in adults[9,10], and comparable associations have been observed in a few studies in children and infants[11,12].

The effects of *n*-3 LCPUFA on the plasma lipid profile may involve changes in the efficiency of lipid transport and apoE-mediated lipid uptake in the tissues[13]. The *APOE4* variant of the gene that encodes apoE has been associated with higher plasma LDL-cholesterol and risk of CHD, whereas the rare *APOE2* variant has been shown to reduce receptor binding and to be associated with lower LDL-cholesterol[14,15]. However, few studies have examined the responsiveness of carriers of the *APOE* variants to *n*-3 LCPUFA intervention[4].

Abbreviations: FADS, fatty acid desaturase; LCPUFA, long-chain PUFA; M, major allele; m, minor allele.

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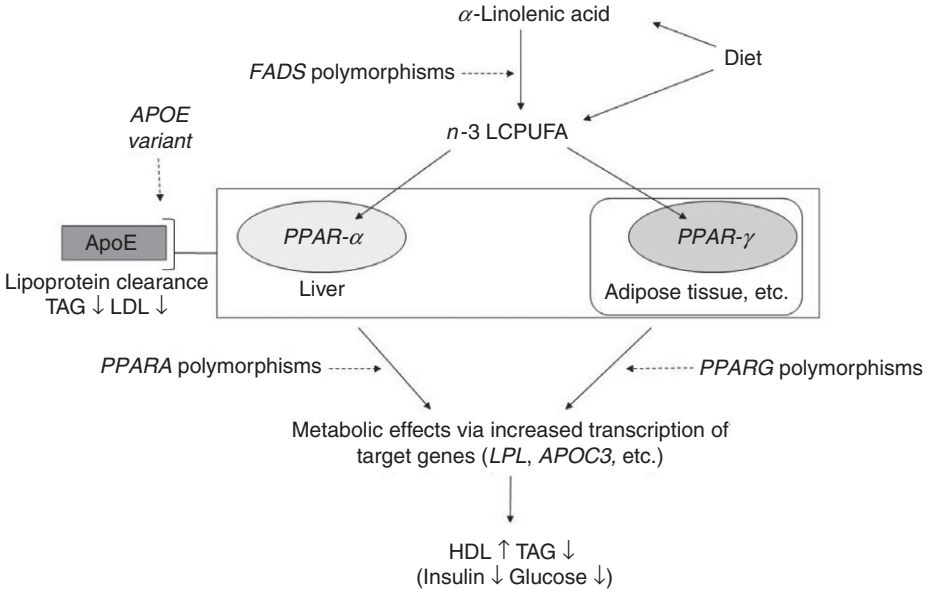

Fig. 1. Outline of the idea of the paper. Conversion of dietary α-linolenic acid to *n*-3 long-chain PUFA (LCPUFA) involves the desaturase enzymes encoded by the fatty acid desaturase (*FADS*) genes and the enzyme's activity (and thereby the conversion) are affected by SNP in *FADS*. *n*-3 LCPUFA may also be consumed preformed in fish and fish oils and are ligands for transcription factors such as *PPAR-α* and *PPAR-γ*. These are found mainly in the liver and in adipose tissue, respectively, and are involved in the effects of *n*-3 LCPUFA on circulating HDL-cholesterol, TAG and markers of glucose homoeostasis. SNP in their genes *PPARA* and *PPARG* affect transcription of target genes thereby probably modulating the effects of *n*-3 LCPUFA. Genetic variants of the apoE gene *APOE* affect lipoprotein clearance, but the effects in combination with *n*-3 LCPUFA are not clear.

Experiments in rodents show that *n*-3 LCPUFA intake can reduce plasma insulin and glucose[16–19], whereas randomised trials in humans show no or inconsistent effects on glucose homoeostasis markers[20–24]. The inconsistencies may be due to genotypic differences, since emerging evidence suggest that the effects of *n*-3 LCPUFA depend on polymorphisms in genes that encode proteins involved in the metabolic pathways or mechanisms of action of these fatty acids[4]. We recently found that fish oil supplementation only reduced plasma glucose in infants who were minor allele carriers of the rs1801282 SNP in the *PPAR*-γ2 gene *PPARG2*[2]. *PPAR*-γ2 is mainly expressed in adipose tissue where it regulates adipogenesis, lipid metabolism and insulin sensitivity[25], whereas *PPAR-α* is a major regulator of lipid metabolism in the liver[26] and both *n*-3 LCPUFA and eicosanoids are ligands for these transcription factors. The minor allele of rs1800206 in the *PPARA* gene has been shown to increase LDL-cholesterol[27] and to modify associations between PUFA intake and the lipid profile in adults[26,28]. However, its effect on gene transcription may depend on the concentration of ligand[27].

In a cross-sectional study among 713 Danish 8–11-year-old children participating in the OPUS School Meal Study, we previously showed that whole-blood EPA was inversely associated with plasma TAG and positively with LDL- and HDL-cholesterol, whereas DHA was inversely associated with serum insulin[29]. In the same cohort, we recently found that *FADS* minor alleles modulated the children's LCPUFA status, that is, minor allele carriers of rs174448 had lower *n*-3 LCPUFA and arachidonic acid in whole blood, whereas carriers of rs1535 minor allele mainly had lower arachidonic acid[5]. In the present study, we aimed to explore (1) whether *FADS* polymorphisms were associated with TAG, HDL- and LDL-cholesterol, insulin and glucose in order to get insight into the role of these genotypes in children's cardiometabolic health and (2) whether polymorphisms in *PPAR* genes and *APOE* modified associations between *FADS* or *n*-3 LCPUFA and the cardiometabolic blood markers, as outlined in Fig. 1. This may help us understand the extent to which PPAR and apoE mediate the effects of *n*-3 LCPUFA and if some children benefit more from a given *n*-3 LCPUFA status than others.

Methods

Study design and participants

The study was based on cross-sectional baseline data from the Optimal well-being, development and health for Danish children through a healthy New Nordic Diet (OPUS) School Meal Study, which investigated the effects of Nordic school meals on children's cardiometabolic health, well-being and cognitive performance[30]. It was conducted according to the guidelines in the Declaration of Helsinki, approved by the Danish National Committee on Biomedical Research Ethics (no. H-1-2010-124), and the baseline study was registered at clinicaltrials.gov as NCT01577277. All children from third and fourth grade at nine schools in different socio-economic areas in the Eastern part of Denmark were invited to participate in the study, and baseline assessments were performed from August to December 2011. Schools were invited by telephone and included if they had at least four classes at the third and fourth grade level, suitable kitchen facilities available for school meal production and high motivation for participation as determined by the study team[30]. Children were excluded only if they had severe food-related

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allergies, food intolerances, or malabsorption, severe mental handicaps or were participating in other research projects that involved blood sampling or radiation. The original power calculation for the OPUS School Meal Study showed that 673 completing children were needed in order to detect a 0·32 point intervention difference in the primary outcome, a metabolic syndrome score[31]. A total of 1021 children were invited of which parents of 834 children (82 %) gave informed written consent to their child's participation[30]. The current paper is based on data from the 757 children who had available data on at least one of the investigated SNP and at least one of the cardiometabolic markers.

Sociodemographics and pubertal status

Parental education was evaluated as the highest education level in the household, based on interview with the parents, and children's ethnicity was characterised as Caucasian, Asian, African American or Latin American by the investigators. Pubertal status was self-evaluated by the child with parental assistance if necessary, using five categories (Tanner stages) of breast development in girls and pubic hair in boys[32].

Blood sampling and anthropometry

Blood sampling and anthropometry were performed by standard procedures in the morning in an air-conditioned double-decker truck equipped as a mobile laboratory. All children reported to have fasted overnight except for twenty-two children of whom nine had only consumed chewing gum or single bites of food. Local anaesthetic patches (EMLA; Astra Zeneca) were provided, and venous blood was drawn from the antecubital vein. Height was measured three times to the nearest 0·1 cm using a portable stadiometer (CMS Weighing Equipment), with the children holding their heads in the Frankfurt horizontal plane, and the mean was calculated. Body weight was measured to the nearest 0·1 kg on a digital scale (Tanita 800S; Tanita) with the child in underwear and without shoes. Sex- and age-adjusted *z*-scores for BMI were calculated using WHO AnthroPlus software[33], and the prevalence of underweight, overweight and obesity was determined based on age- and sex-specific cut-offs as described by Cole *et al.*[34,35].

Blood analyses

Glucose was assessed in fresh blood by a Hemocue Glucose 201 (Hemocue Denmark) calibrated to calculate plasma concentrations from whole blood. Blood for insulin analysis was collected in serum tubes with gel and left to coagulate for 30 min at room temperature. Serum and heparinised plasma for measurement of cholesterols and TAG were obtained by centrifugation at 2500 ***g*** for 10 min. Heparinised whole blood was mixed with 0·1 % butylated hydroxytoluene (Sigma-Aldrich) in ethanol (0·1 ml per ml blood) for fatty acid composition determinations. All blood fractions were stored at –80°C until analysis.

Serum insulin was measured by an automated chemiluminescent immunoassay on an ADVIA Centaur XP (Siemens Healthcare). Plasma total and HDL-cholesterol and TAG were measured on a Vitros 5.1 FS (Ortho-Clinical Diagnostics). LDL-cholesterol concentrations were calculated by Friedewald's equation[36]. The inter- and intra-assay CV were 1·4 and 1·2 % (total cholesterol); 2·0 and 1·2 % (HDL-cholesterol); 1·5 and 0·8 % (TAG); and 2·5 and 3·1 % (insulin).

Whole-blood fatty acid composition was measured by high-throughput GC within 3 months after blood sampling as previously described[29,37,38]. Briefly, thirty-two individual fatty acids were determined quantitatively by comparison with internal (22 : 3*n*-3 ethyl ester; Nu-Check Prep) and external reference standards (GLC-462, Nu-Check Prep). Lipids were extracted using chloroform:methanol (2:1, v/v) and transesterified to fatty acid methyl esters using 14 % boron trifluoride in methanol prior to GC analyses with flame ionisation detection using a Varian 3900 gas chromatograph equipped with a DB-FFAP 15 m × 0·10 mm injected dose × 0·10 µm film thickness capillary column (J&W Scientific from Agilent Technologies). *n*-3 LCPUFA were calculated as EPA + DHA in % of the total fatty acids. The intra- and inter-assay CV were 1·3 and 4·5 % for EPA and 2·4 and 6·4 % for DHA, respectively.

Genotyping and selection of SNP

Buffy coat DNA extraction and genotyping were performed at LGC Genomics Ltd using their Competitive Allele Specific Polymerase Chain Reaction genotyping technology. We included six functional SNP that have been associated with either blood LCPUFA, lipid profile or glucose metabolism and/or were regulated by LCPUFA in previous studies[2,6,13,26,28]: *FADS* rs1535 and rs174448, *PPARA* rs1800206, *PPARG2* rs1801282, as well as *APOE* rs7412+rs429358 which combined determine *APOE* genotype[4] (online Supplementary Tables S1 and S2).

Statistical analysis

Data were analysed with SPSS version 23 (IBM Corporation) and R version 3.5.1 (The R Foundation for Statistical Computing Platform), and statistical significance was established at $P < 0{\cdot}05$. Hardy–Weinberg equilibrium was assessed by χ^2 test[39] and linkage disequilibrium by use of the R package 'genetics'. Characteristics of included and non-included children were compared using unpaired *t* test (age and BMI *z*-score) or χ^2 test (sex and parental education), and allele frequencies were compared between ethnic groups by χ^2 test. Cardiometabolic blood markers (plasma TAG, HDL-cholesterol, LDL-cholesterol and glucose and serum insulin) were checked for normal distribution by visual inspection of qq-plots and histograms, and TAG and insulin were ln-transformed to obtain normality. For each SNP, children were categorised as major allele homozygous (MM) or minor allele carriers (Mm+mm). We generated *APOE* genotypes based on the following rs7412/rs429358 allele combinations: mm/MM (*E2/E2*), Mm/MM (*E3/E2*), MM/MM (*E3/E3*), MM/Mm (*E3/E4*), MM/mm (*E4/E4*) and Mm/Mm (*E2/E4*) (online Supplementary Table S2). None of the children was carriers of *E1*. In order to assess the effects of *E2* and *E4* carriage compared with the more common *E3/E3* genotype, we excluded twenty-one children with *E2/E4* and pooled *E3/E2* + *E2/E2* as well as *E3/E4* + *E4/E4*. Cardiometabolic characteristics of the *APOE* genotypes were compared by one-way ANOVA.

To test if the *FADS* SNP were associated with the cardiometabolic outcomes, we fitted linear mixed models including school and class as random effects, and age, height and BMI *z*-score as fixed effects or covariates, in order to reduce variation. Puberty and sex were included in the models as a sex × puberty interaction term, due to the different puberty scales used for boys and girls. Linear mixed models were fitted to test whether the *PPAR* SNP (rs1801282 or rs1800206) or *APOE* genotype showed interaction with the *FADS* SNP (rs1535 or rs174448), or with whole-blood *n*-3 LCPUFA. For all of the outcomes, these models included one interaction term at a time as well as the same adjustments as described above. Each statistical model included only participants with available data on the specific SNP and outcome. To check for consistency, all analyses were also conducted without any adjustments, in secondary analyses.

Results

Children's characteristics

There was an even distribution of boys and girls, most children were of Caucasian origin and had normal weight, and about 1/3 (mainly girls) had entered puberty (Table 1). The 757 included children comprised 91 % of the original OPUS School Meal Study population and did not differ from the non-included children with regard to age, sex, BMI *z*-scores or parental education ($P > 0{\cdot}24$, data not shown). The minor allele frequencies of the six investigated SNP ranged from 6 to 36 % and showed no deviations from Hardy–Weinberg Equilibrium (online Supplementary Table S1). As expected, the two FADS SNP (rs1535 and rs174448) were somewhat in linkage disequilibrium ($D = 0{\cdot}015$, r^2 0·43), but all other SNP combinations showed little

Table 1. Baseline characteristics of the 757 children in the study (Mean values and standard deviations; median values and interquartile ranges (IQR); percentages)

	Descriptive statistics	
	Mean	SD
Male sex (%)	53	
Caucasian (%)*	95	
Entered puberty (yes) (%)	34	
Age (years)	10·0	0·6
Height (cm)	142·6	7·1
BMI-for-age *z*-score	0·15	1·08
BMI category (% uw/nw/ow/ob)	10/76/12/2	
TAG (mmol/l)		
Median	0·61	
IQR	0·50–0·77	
Total cholesterol (mmol/l)	4·09	0·65
HDL-cholesterol (mmol/l)	1·44	0·30
LDL-cholesterol (mmol/l)	2·34	0·57
Glucose (mmol/l)	5·22	0·46
Insulin (pmol/l)		
Median	43	
IQR	31–59	
Whole-blood fatty acids (%)		
Arachidonic acid (20 : 4*n*-6)	9·21	1·23
EPA (20 : 5*n*-3) + DHA (22 : 6*n*-3)	3·59	0·96

uw, Underweight; nw, normal weight; ow, overweight; ob, obese.
* The remaining children were of Asian (2 %), African American (2 %) and Latin American (1 %) origin.

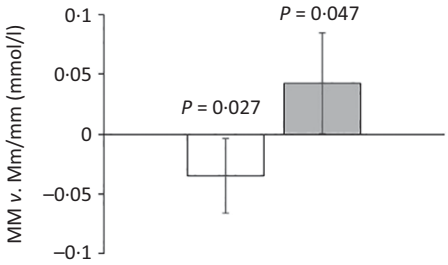

Fig. 2. Estimated differences in TAG and HDL-cholesterol between major allele homozygotes and minor allele carriers of the fatty acid desaturase (*FADS*) rs174448 genotype. Bars and error lines indicate estimated differences and 95 % confidence intervals, respectively, for MM compared with Mm/mm adjusted for age, sex × puberty, height, BMI *z*-score, school and class (n 316 for MM and n 440 for Mm/mm). *P* values are shown for these comparisons. □, TAG; ▩, HDL.

indication of linkage (D between –0·001 and 0·008, $r^2 < 0{\cdot}006$). There were no differences in allele frequencies between ethnic groups for any of the SNP ($P > 0{\cdot}19$).

Associations between FADS genotypes and the cardiometabolic markers

Major allele homozygotes of rs174448 had 0·04 (95 % CI 0·00, 0·07) mmol/l lower plasma TAG and 0·04 (95 % CI 0·00, 0·09) mmol/l higher HDL-cholesterol than minor allele carriers (Fig. 2). Comparable effect sizes in the same direction were seen for rs1535, but these did not reach statistical significance (online Supplementary Table S3). In addition, minor allele carriers of both rs174448 and rs1535 tended to have lower plasma glucose ($P < 0{\cdot}10$, online Supplementary Table S3). There were no other differences, and unadjusted analyses showed the same results (data not shown).

Effect modification by PPAR SNP

None of the associations between FADS and the cardiometabolic markers was modified by rs1800206 (*PPARA*) or rs1801282 (*PPARG2*) (all $P_{interaction} > 0{\cdot}10$, data not shown). Furthermore, there were no interaction between *n*-3 LCPUFA and the *PPARA* SNP (online Supplementary Table S4). However, as shown in Table 2, the associations between *n*-3 LCPUFA and TAG, LDL-cholesterol and insulin were modified by *PPARG2* genotype. The associations in minor allele carriers were inverse, whereas there were no or positive associations in major allele homozygotes (Table 2). There was no interaction for HDL-cholesterol or glucose (Table 2). Repeating the analyses without adjustments did not change the results (data not shown).

Effect modification by APOE genotypes

As expected, compared with children with the most common genotype *E3/E3*, those with *E3/E2* + *E2/E2* had lower LDL-cholesterol (1·92 (SEM 0·05) *v.* 2·35 (SEM 0·03) mmol/l) and higher HDL-cholesterol (1·52 (SEM 0·03) *v.* 1·44 (SEM 0·01)), whereas children carrying *E3/E4* + *E4/E4* had higher LDL-cholesterol of 2·55 (SEM 0·04) mmol/l (all $P < 0{\cdot}05$). *APOE* genotype modified the association between *FADS* rs1535 and HDL-cholesterol ($P_{rs1535 \times APOE} = 0{\cdot}019$), as rs1535 major allele homozygotes

Table 2. Associations between *n*-3 long-chain PUFA (LCPUFA) and the cardiometabolic markers according to *PPARG2* rs1801282 genotype* (*β* Coefficients and 95 % confidence intervals)

	MM (*n* 562)		Mm/mm (*n* 180)		
Cardiometabolic marker	*β*	95 % CI	*β*	95 % CI	*P* interaction$_{n\text{-}3\ \text{LCPUFA} \times \text{rs180182}}$†
TAG (ln mmol/l)	–0·005	–0·034, 0·024	–0·110	–0·157, –0·064	<0·001
HDL-cholesterol (mmol/l)	0·009	–0·017, 0·034	0·015	–0·028, 0·057	0·810
LDL-cholesterol (mmol/l)	0·084	0·036, 0·133	–0·078	–0·158, 0·003	<0·001
Glucose (mmol/l)	–0·029	–0·069, 0·010	–0·021	–0·086, 0·043	0·830
Insulin (ln pmol/l)‡	–0·023	–0·059, 0·014	–0·123	–0·182, –0·063	0·005

M, major allele; m, minor allele.
* Estimates are adjusted slopes (*β*) and 95 % in the given units per % *n*-3 LCPUFA in whole blood; insulin and TAG were ln-transformed, and their estimates are given on a logarithmic scale. *n*-3 LCPUFA is given as the sum of EPA and DHA (%) in whole blood.
† *P* for interaction between *n*-3 LCPUFA and *PPARG2* rs180182 in linear mixed models adjusted for age, sex × puberty, height, BMI *z*-score, school and class.
‡ *n* 534 for MM and *n* 173 for Mm/mm.

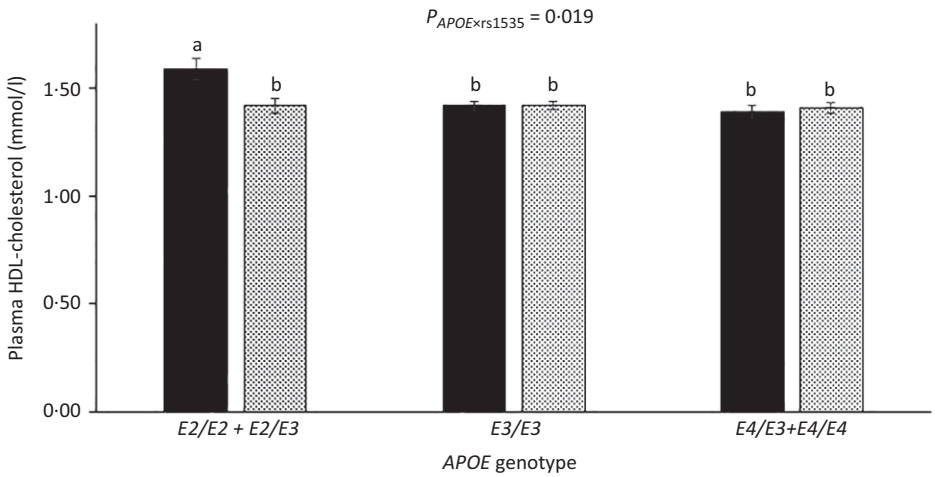

Fig. 3. HDL-cholesterol in the children according to fatty acid desaturase (*FADS*) rs1535 genotype and *APOE* genotype. Bars and error lines indicate raw means with their standard errors, respectively. *P* values are shown for rs1535 × *APOE* interaction. [a,b] Unlike letters indicate pairwise differences based on a linear mixed model adjusted for age, sex × puberty, height, BMI *z*-score, school and class (*n* 713). ■, rs1535 MM; ▨, rs1535 Mm/mm.

Table 3. Associations between *n*-3 long-chain PUFA (LCPUFA) and the cardiometabolic markers according to *APOE* genotype* (*β* Coefficients and 95 % confidence intervals)

	E2/E2 + *E2/E3* (*n* 100)		*E3/E3* (*n* 410)		*E4/E3* + *E4/E4* (*n* 193)		
Cardiometabolic marker	*β*	95 % CI	*β*	95 % CI	*β*	95 % CI	*P* interaction$_{n\text{-}3\ \text{LCPUFA} \times \textit{APOE}}$†
TAG (mmol/l)	–0·049	–0·115, 0·017	–0·028	–0·062, 0·006	–0·033	–0·079, 0·013	0·855
HDL-cholesterol (mmol/l)	0·068	0·009, 0·127	–0·000	–0·030, 0·029	0·003	–0·038, 0·044	0·115
LDL-cholesterol (mmol/l)	–0·006	–0·113, 0·101	0·060	0·007, 0·114	0·064	–0·010, 0·138	0·519
Glucose (mmol/l)	–0·037	–0·127, 0·053	–0·050	–0·096, –0·004	0·010	–0·053, 0·073	0·313
Insulin (pmol/l)‡	–0·094	–0·178, –0·010	–0·038	–0·081, 0·005	–0·048	–0·108, 0·011	0·499

* Estimates are adjusted slopes (*β*) and 95 % per % *n*-3 LCPUFA in whole blood; insulin and TAG were ln-transformed, and their estimates are given on a logarithmic scale. *n*-3 LCPUFA is given as the sum of EPA and DHA (%) in whole blood. Twenty-one children with *E2/E4* were excluded from the analysis.
† *P* for interaction between *n*-3 LCPUFA and *APOE* in linear mixed models adjusted for age, sex × puberty, height, BMI *z*-score, school and class.
‡ *n* 95 for *E2/E2* + *E2/E3*, *n* 391 for E3/E3 and *n* 183 for *E4/E3* + *E4/E4*.

carrying *APOE2* had higher HDL-cholesterol than all other genotype combinations (Fig. 3). There were no interactions between whole-blood *n*-3 LCPUFA and *APOE* genotype (Table 3). However, when looking at the two *n*-3 LCPUFA separately, DHA was positively associated with HDL-cholesterol only among children with *APOE2* ($P_{n\text{-}3\ \text{LCPUFA} \times \textit{APOE}} = 0{\cdot}04$, data not shown). The unadjusted models showed the same overall results (data not shown).

Discussion

The present study showed that children carrying two *FADS* rs1174448 major alleles, who have previously been shown to have higher blood *n*-3 LCPUFA[5], had lower plasma TAG and higher HDL-cholesterol than minor allele carriers. Major allele homozygotes of *FADS* rs1535 also had higher HDL-cholesterol, but only when also carrying *APOE2*. In contrast, associations between

whole-blood *n*-3 LCPUFA and the cardiometabolic markers did not seem to depend on *APOE* genotype. There were no interactions between *n*-3 LCPUFA status and *PPARA* genotype, but *n*-3 LCPUFA was associated with lower TAG and insulin in carriers of *PPARG2* minor allele, which has been shown to reduce transcription, and positively associated with LDL-cholesterol in major allele homozygotes. These data could indicate that endogenous as well as exogenous *n*-3 LCPUFA can modulate children's cardiometabolic profile and that *PPARγ*2 and apoE play some role in their mechanisms of action, thereby suggesting that potential benefits of *n*-3 LCPUFA consumption may differ between children dependent on their genotype.

FADS rs174448 was associated with plasma TAG and HDL-cholesterol, and this was also indicated, but less pronounced, for rs1535. FADS catalyse the endogenous conversion of short- to long-chain PUFA of both the *n*-3 and *n*-6 family, and the SNP generally encode desaturase enzymes with reduced activity. In the children of the present cohort and among infants, we have seen that minor allele carriage of rs174488 was associated with low *n*-3 LCPUFA status and a tendency of low arachidonic acid (20 : 4*n*-6)[5,6], whereas minor allele carriage of rs1535 was only associated with low arachidonic acid in the children[5] and even with high DHA in the infants[6]. This indicates that the associations between rs174448, TAG and cholesterol in the present study could be driven by reduced *n*-3 LCPUFA status in the minor allele carriers. This would fit with results from numerous trials showing that fish oil supplementation lowers plasma TAG and increases HDL-cholesterol[40]. The estimated 0·04 mmol/l lower TAG and higher HDL-cholesterol in the rs174448 major allele homozygotes compared with minor allele carriers correspond to 50–100 % of the changes that we found in a recent randomised trial in 8–9-year-old children in response to intervention with oily fish several times per week[41].

Few studies have investigated associations between *FADS* SNP, TAG and cholesterol in children. Standl *et al.*[11] found that minor allele carriage of some other *FADS* SNP was associated with high plasma TAG and low HDL in 10-year-old German children. In contrast, in our infant study, rs174448 minor allele was not associated with TAG, but with lower LDL-cholesterol in all infants as well as with lower HDL-cholesterol among breastfed infants only[12]. In line with our results, previous studies in adults have also reported associations between *FADS* minor alleles and higher TAG, as well as lower HDL-cholesterol[42,43]. We found that HDL-cholesterol was higher in children carrying *APOE2* and two rs1535 major alleles compared with all other genotype combinations. *n*-3 LCPUFA may increase reverse cholesterol transport via activation of hepatic transcription factors[44], and *APOE2* has been associated with slower catabolism of HDL particles[45], which may result in higher circulating HDL-cholesterol when combined with higher LCPUFA. However, we did not find interaction between *APOE* genotype and *n*-3 LCPUFA on HDL-cholesterol or any other associations with the cardiometabolic markers, although DHA was positively associated with HDL-cholesterol only among children with *APOE2*, when the two *n*-3 LCPUFA were analysed separately.

Among the blood lipid outcomes, the *PPARG2* SNP rs1801282 modified associations between *n*-3 LCPUFA and TAG and LDL-cholesterol. The observed positive association between *n*-3 LCPUFA and LDL-cholesterol among *PPARG2* rs1801282 major allele homozygotes was not seen in our infant fish oil trial[2] or, to our knowledge, in previous trials in adults. For TAG, inverse associations were only seen in the minor allele carriers, which is in agreement with the *PPARG2*-dependent effects of fish oil supplementation in our previous infant study[2] and in studies in adults[46,47]. The rs1801282 minor allele has been shown to reduce transcription[48], and we hypothesise that increased levels of *PPAR-γ*2 ligands such as EPA and DHA may have a larger impact on the transcription of target genes (such as the lipoprotein lipase gene), when PPAR-γ2 is less efficient. Besides lipid metabolism, *PPAR-γ* is also important in the regulation of genes involved in glucose metabolism[49] and animal studies have shown that *n*-3 LCPUFA may mediate effects on circulating glucose through *PPAR-γ*[50,51]. We found no interaction between *FADS* or *n*-3 LCPUFA on plasma glucose, but that rs1801282 modified the association between *n*-3 LCPUFA and serum insulin with an inverse association in minor allele carriers only. This concurs with the results of our previous infant trial, where fish oil reduced plasma glucose in carriers of rs1801282 minor allele only[2] and comparable interactions were indicated in a cross-sectional study among Finnish adults[52]. Such a genotype-dependent effect of *n*-3 LCPUFA may explain why only some studies have shown effects of fish oil supplementation on glucose homoeostasis, but needs replication.

The magnitude of the estimated genotype-specific effect in the present study corresponds to a reduction in TAG and insulin of about 0·11 mmol/l and 8 pmol/l, respectively, among *PPARG2* minor allele carriers and no change in major allele homozygotes, if all children's whole-blood *n*-3 LCPUFA is increased from 3·5 to 5·0 %. This could be achieved by consumption of oily fish several times per week as demonstrated in our recent fish trial[53]. If true, such a genotypic difference would likely be of relevance for long-term cardiometabolic health. Our results did not indicate that the *PPARA* SNP rs1800206 was a potential effect modifier. Previous cross-sectional studies in adults have shown that rs1800206 modified the association between total PUFA intake and plasma TAG[26] and HDL-cholesterol[28]. We cannot rule out the possibility that the low minor allele frequencies of rs1800206 and limited sample size of the present study may have compromised our ability to detect any effect modifications by this SNP.

The present study is limited by its cross-sectional design, which does not allow for inferences about causality, and by the reduced power in especially the genotype interaction analyses. The study is merely hypothesis generating and needs replication in larger studies. It is however strengthened by the targeted approach, the considerable sample size and the use of the whole-blood *n*-3 LCPUFA biomarker, which reflects dietary intake of recent weeks[54]. Due to the exploratory nature of the study and since the outcome variables correlated, we did not adjust for multiple testing, but interpreted our findings with caution and focus on consistency, and the results were highly consistent in adjusted and unadjusted analyses. The original power calculation for the study was based on a metabolic

syndrome score assessed after a school meal intervention, so some of the results could be chance-findings. Due to the low number of minor allele homozygotes, we pooled the minor allele homozygotes and heterozygotes in the analyses, which is a commonly used approach[11], even without certainty that the minor allele was acting in a dominant fashion, that is, that the number of minor alleles is less relevant. Although genotypes are less prone to confounding than, for example, measures of dietary intake, they may be confounded by genetic ancestry, which we did not correct for. However, this is unlikely to be a major confounder in our study, since the study population was highly homogeneous with 95 % of the children being of white, Caucasian origin. Another potential confounder, which we did not control for, is socio-economic status; however, as previously described[29], most of the children came from households with a higher education. Finally, the study sample was to a large degree representative of Danish children of this age[29] and there were no apparent differences between the children, who were included and not included, in the present study. However, other studies would be needed to verify if the demonstrated associations could be found among populations with different demographics such as ethnic backgrounds, rates of overweight and dietary habits.

In conclusion, this study among Danish children showed that the least common allele of *FADS* rs174448 was associated with high TAG and low HDL-cholesterol, whereas those with two *FADS* rs1535 major alleles had high HDL-cholesterol, but only if they were *APOE2* carriers. *APOE* genotype did not modify the beneficial associations between *n*-3 LCPUFA and TAG, LDL-cholesterol and insulin, but these associations were mainly seen in children with the variant genotype of *PPARG2*. These results indicate that (*n*-3) LCPUFA may affect children's cardiometabolic profile in a potential genotype-specific manner, which may suggest that some children could benefit more from *n*-3 LCPUFA consumption than others. However, due to the cross-sectional design and limited power, the results need replication in larger genetic studies and in randomised trials, which examine the combined effect of *n*-3 LCPUFA and genotype on children's cardiometabolic health.

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C. T. D., S. V. and L. L. designed the research; C. T. D. led the data collection; K. D. S. was responsible for the fatty acid analyses; S. V. performed the statistical analysis; C. T. D. wrote the paper and had primary responsibility for the final content. All authors read and approved the final manuscript.

None of the authors has any conflicts of interest to declare.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520002822

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