European Journal of Clinical Nutrition (2017), 1–9

www.nature.com/ejcn

ORIGINAL ARTICLE

Associations between fatty acids and low-grade inflammation in children from the LISAplus birth cohort study

C Harris[1], H Demmelmair[2], A von Berg[3], I Lehmann[4,5], C Flexeder[1], B Koletzko[2], J Heinrich[1,6] and M Standl[1] for the LISAplus study group[7]

BACKGROUND/OBJECTIVES: Assessing fatty acid (FA) composition in relation to inflammatory markers can shed light on the role of different FA and their metabolism in low-grade inflammation. Existing exploratory studies in children are scarce, and findings inconsistent. We hence aim to analyse associations of FA with common inflammatory markers, high-sensitivity C-reactive protein (hs-CRP) and interleukin-6 (IL-6), in 10-year-old children.

SUBJECTS/METHODS: Complete data were available for 958 participants from the 10-year follow-up of the LISAplus (Influence of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood plus the Influence of Traffic Emissions and Genetics) birth cohort study. FA composition was assessed in serum glycerophospholipids. Hs-CRP and IL-6 were categorised into three levels. Associations of FA with inflammatory markers were assessed using multinomial logistic regression, adjusting for potential confounders. Additionally, sex-stratified analyses were carried out.

RESULTS: FA exposures associated with significantly higher low-grade inflammation, as indicated by higher hs-CRP or IL-6 levels, included: palmitic acid (PA) (IL-6: $P < 0.001$, 95% confidence interval: 1.30; 2.43), arachidonic acid (AA) (hs-CRP: $P = 0.002$, 1.07; 1.31), n-6 highly unsaturated FA (HUFA) (hs-CRP: $P = 0.002$, 1.06; 1.27), ratio of AA to linoleic acid (AA/LA) (hs-CRP: $P < 0.001$, 1.16; 1.62) and total saturated FA (SFA) (IL-6: $P < 0.001$, 1.77; 3.15). FA exposures associated with reduced levels of inflammatory markers included LA (hs-CRP: $P = 0.001$, 0.84; 0.96; IL-6: $P < 0.001$, 0.69; 0.90) and total polyunsaturated FA (PUFA) (IL-6: $P < 0.001$, 0.57; 0.78).

CONCLUSIONS: These findings suggest that higher SFA and minor n-6 HUFA, namely PA and AA, are associated with increased low-grade inflammation in children, whereas the major dietary n-6 PUFA and total PUFA are associated with reduced inflammation. Elevated desaturase activity, estimated by the ratio AA/LA, may be associated with higher inflammation, particularly in boys.

European Journal of Clinical Nutrition advance online publication, 7 June 2017; doi:10.1038/ejcn.2017.73

INTRODUCTION

A state of chronic low-grade inflammation, characterised by raised concentrations of circulating inflammatory markers, is known to underlie metabolic conditions such as atherosclerosis[1,2] and obesity.[3,4] C-reactive protein (CRP) is an acute phase protein synthesised primarily in response to circulating proinflammatory cytokine interleukin-6 (IL-6).[5] Elevated concentrations of both these inflammatory markers have been observed in association with arterial changes in children,[6,7] suggesting a possible role of low-grade inflammation in the pathogenesis of early atherosclerosis.

Some dietary components have the capacity to influence inflammatory processes,[8] thereby signifying potential modifiable targets for the prevention of low-grade inflammation and associated diseases. It is now recognised that lipid-derived mediators, produced from long-chain fatty acids (FAs), are greatly involved in the metabolic mechanisms of inflammation.[9] Long-chain n-3 polyunsaturated FA (PUFA) have been shown to have anti-inflammatory properties, partly by reducing the levels of arachidonic acid (AA), a known source of proinflammatory eicosanoids in immune cell membranes.[10] FA composition, often measured in plasma or serum lipids,[11,12] reflects both dietary FA intake and endogenous FA metabolism.[13] Especially the major dietary n-6 PUFA, linoleic acid (LA), and the long-chain n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are well reflected in serum phospholipids. A number of studies analysing FA composition in relation to inflammatory markers in adults have shed light on the possible involvement of different FA and their metabolism in low-grade inflammation.[14–17] Evidence on the relationship between FA composition and low-grade inflammation in children is however limited to few studies with inconsistent findings.[18,19] Despite their valuable contributions, there is still insufficient evidence to draw definitive conclusions regarding FA in the modulation of inflammatory processes in children.

Therefore, the aim of this exploratory study was to analyse the associations between different FA measured in serum glycerophospholipids assumed to have relevant roles in inflammatory processes, with common markers of inflammation in 10-year-old children, namely high-sensitivity CRP (hs-CRP) and IL-6.

[1]Institute of Epidemiology I, Helmholtz Zentrum München—German Research Centre for Environmental Health, Neuherberg, Germany; [2]Ludwig Maximilians-Universität München, Dr von Hauner Children's Hospital, Munich, Germany; [3]Department of Pediatrics, Research Institute, Marien-Hospital, Wesel, Germany; [4]Core Facility 'Studies', Helmholtz Zentrum für Umweltforschung UFZ, Leipzig, Germany; [5]Department of Environmental Immunology, Helmholtz Zentrum für Umweltforschung UFZ, Leipzig, Germany and [6]Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Inner City Clinic, University Hospital of Munich (LMU), Munich, Germany. Correspondence: Dr M Standl, Institute of Epidemiology I, Helmholtz Zentrum München—German Research Center for Environmental Health, Ingolstädter Landstrase 1, Neuherberg 85764, Germany.
E-mail: marie.standl@helmholtz-muenchen.de
[7]Members of the LISAplus study group are listed before references.
Received 16 August 2016; revised 10 April 2017; accepted 13 April 2017

MATERIALS AND METHODS

Data were obtained from the 10-year follow-up assessment of the ongoing LISAplus (Influence of Lifestyle-Related Factors on the Immune System and the Development of Allergies in Childhood plus the Influence of Traffic Emissions and Genetics) birth cohort study.[20] The study design, recruitment and exclusion criteria have been described previously.[20] In brief, between the end of 1997 and beginning of 1999, healthy full-term newborns were recruited from obstetric clinics within four German cities. Information was collected using identical questionnaires and at physical examinations. During the 10-year follow-up physical examination, venous blood samples were collected in serum separator tubes and centrifuged at 3000 U/min for 10 min at 4 °C. Serum was aliquoted and stored at − 80 °C for later analysis of fatty acids and inflammatory markers.

Approval by the local ethics committee (Bavarian Board of Physicians, University of Leipzig, Board of Physicians of North-Rhine-Westphalia) and written consent from participants' families were obtained.

Inflammatory markers: hs-CRP and IL-6

Serum concentrations of hs-CRP were measured using the Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitive assay, and concentrations of IL-6 were measured by flow cytometry using a cytometric bead array (BD CBA Human Soluble Flex Set system; Becton Dickinson, Heidelberg, Germany), according to the manufacturer instructions. Measured hs-CRP and IL-6 concentrations were highly skewed, with many observations below the detection limit. Given this non-normal distribution, data categorisation was required for analyses. Both inflammatory markers were hence categorised into three levels separately for girls and boys, considering all children with available measurements ($n = 1083$, see Figure 1). Categories of hs-CRP were defined similarly to those published in the recent study on fatty acids and hs-CRP in European children[19] to ease comparison: (I) hs-CRP < 0.02 mg/dl; (II) hs-CRP $\geqslant 0.02$ mg/dl and $<$ 75th sex-specific percentile of those with hs-CRP $\geqslant 0.02$ mg/dl (< 0.11 mg/dl in girls; < 0.09 mg/dl in boys); and (III) hs-CRP $\geqslant$ 75th sex-specific percentile of those with hs-CRP $\geqslant 0.02$ mg/dl ($\geqslant 0.11$ mg/dl in girls; $\geqslant 0.09$ mg/dl in boys). IL-6 was categorised with reference to the minimal detectable concentration (1.5 pg/ml): (I) IL-6 $\leqslant$ 1.5 pg/ml; (II) IL-6 $>$ 1.5 pg/ml and $<$ 75th sex-specific percentile of those with IL-6 $>$ 1.5 pg/ml ($<$ 4.26 pg/ml in girls; $<$ 3.93 pg/ml in boys); and (III) IL-6 $\geqslant$ 75th sex-specific percentile of those with IL-6 $>$ 1.5 pg/ml ($\geqslant$ 4.26 pg/ml in girls; $\geqslant$3.93 pg/ml in boys).

Fatty acid status

Serum glycerophospholipid FA concentrations were measured by a high-throughput method developed with plasma samples, and successfully applied previously for analyses of FA in serum from cord blood and blood samples collected at ages 2, 6 and 10 years in the LISAplus study.[21–23] Full details on sample preparation and analysis have been described elsewhere.[24] The following FA were analysed in the present study: palmitic acid (PA), oleic acid (OA), LA, γ-linoleic acid (GLA), dihomo-γ-linoleic acid (DHGLA), AA, α-linoleic acid (ALA), EPA, docosapentaenoic acid (DPA), DHA, total SFA, total monounsaturated FA (MUFA) and total PUFA. Additionally, we included FA groups and ratios that have previously been proposed to have a role in inflammation. Highly unsaturated n-6 and n-3 FA (HUFA: $\geqslant$20 carbons and $\geqslant$3 double bonds) are known precursors of chemical messengers involved in inflammation.[25] Since n-6 and n-3 PUFA compete for the same desaturase enzymes (Δ5 and Δ6 desaturase) for long-chain PUFA synthesis, it has been discussed that a low ratio of n-6 to n-3 PUFA (n-6/n-3) could reduce inflammation by favouring conversion of dietary n-3 PUFA to EPA, and limiting AA availability.[26] On the other hand, this has not been confirmed, and accumulating evidence indicates no role for n-6/n-3 in modulating inflammation.[27,28] EPA and DHA have been reported to inhibit AA metabolism and to form potent anti-inflammatory lipid mediators;[9,10] their respective ratios (EPA/AA and DHA/AA) have also proven relevant in the reduction of inflammatory cytokine release.[29,30] Finally, greater desaturase activity has been suggested to promote inflammation by increasing the availability of eicosanoid precursors.[31] Since practical reasons prevent the measurement of desaturase activity directly, product-to-precursor ratios, such as AA/LA or AA/DHGLA, can be used as surrogate measures to estimate overall and Δ5 desaturase activity, respectively.[32] Full names of abbreviations of exposure variables and the FAs encompassed under umbrella terms (HUFA, n-6/n-3, SFA, MUFA and PUFA) are listed in Supplementary Table S1. For use in our main analyses, proportions of each FA relative to total FA (%FA) were calculated. In an additional sensitivity analysis we analysed FA concentrations.

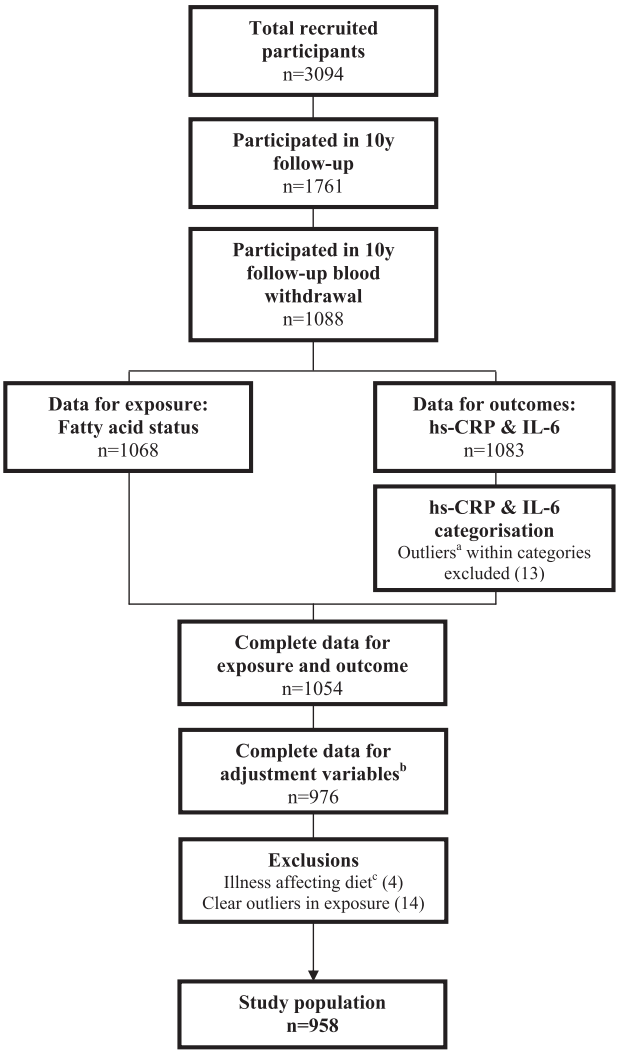

Figure 1. Study participants. [a]hs-CRP $>$ 1 mg/dl or IL-6 $>$ 20 pg/ml. [b]Adjustment variables: sex, region, age, maternal education level, BMI, screen time, onset of puberty and whether the child was ever breastfed. [c]Illness affecting diet: for example, diabetes, anorexia, coeliac disease, cancer.

Adjustment variables

Variables used for adjusting statistical models included sex, recruitment region (Munich; Wesel; Bad Honnef; Leipzig), exact age at physical examination (years), maternal education level (highest level achieved—low: $<$ 10th grade; medium: 10th grade; high: $>$10th grade), BMI (in kg/m^2, calculated from height and

weight measurements obtained at the physical examination), screen time (low: ⩽1 h in winter and ⩽2 h in summer; medium: >1 h in winter or >2 h in summer; high: >1 h in winter and >2 h in summer), onset of puberty (yes: estradiol >18.4 pmol/l in females; testosterone >0.09 nmol/l in males) and whether the child was ever breastfed (yes: ⩾1 month).

Statistical analysis

Differences in characteristics between girls and boys were tested by Student's *t*-test (means) or by Wilcoxon's rank-sum test (medians) for continuous variables, and by Pearson's χ^2 test for categorical variables. A two-sided α-level of 5% was considered significant. Associations of %FA with hs-CRP and IL-6 were assessed using multinomial logistic regression, given that the outcome variables hs-CRP and IL-6 were both categorised into three levels (ordinal logistic regression could not be applied, as the assumption of proportional odds was not satisfied). Results were presented as odds ratios with corresponding 95% confidence intervals (OR (95% CI)), with the lowest level (I) as the reference category. A basic model (M1) and a fully adjusted model (M2) were used, adjusting for: (M1) sex, region, age and maternal education level; and (M2) further adjusting for BMI, screen time, onset of puberty and whether the child was ever breastfed. Sensitivity analyses were run stratified by sex. To avoid chance findings resulting from the large number of regression models, we corrected for multiple testing using Bonferroni correction: the α-level was divided by twenty (the number of tests performed). This yielded a corrected two-sided α-level of 0.0025 (0.05/20 = 0.0025). For sex-stratified analyses, the *P*-value was further divided by two, accounting for the analysis at two levels (0.0025/2 = 0.00125). Finally, we reran our analyses using FA concentrations, including adjustment for total FA. To avoid problems of multicollinearity, we used FA residuals calculated by regressing individual FA concentrations on total FA. All analyses were conducted using R, version 3.3.0 (https://www.R-project.org/),[33] with code available upon request. Multinomial logistic regression was calculated using the multinom() function in package 'nnet'.[34]

RESULTS

Complete information on FA, hs-CRP, IL-6 and adjustment variables was available for 958 participants (Figure 1). Subjects with hs-CRP values >1 mg/dl[35] or IL-6 values >20 pg/ml[36] were considered as outliers and excluded from the analysis (7 subjects with hs-CRP levels from 1.03 to 4.37 mg/dl and 6 subjects with IL-6 levels from 32.9 to 4384.0 pg/ml). Only participants with complete data for both exposure and outcome measurements were included ($n = 1054$). Participants were further excluded who were lacking data for adjustment variables (78 subjects), who reported an illness affecting diet (4 subjects), or presented outlying values in exposure measurements (14 subjects). The resulting sample size ($n = 958$) was considered adequate for multinomial logistic regression analyses, based on reports from simulation studies.[37,38]

Basic characteristics of the study population are displayed in Table 1. About half of the study participants were from Munich with a high maternal education level. Almost all children were breastfed and most reported low screen time. Girls had significantly lower screen time than boys and about two-thirds of them had entered onset of puberty, compared with just around 27% of boys. Girls also had higher hs-CRP and IL-6 levels than boys.

Results from the multinomial logistic regression models (M1: basic model and M2: fully adjusted model) are presented in Table 2. Associations observed in the fully adjusted model (M2) are displayed in Figure 2. FA exposures associated with significantly higher low-grade inflammation, as indicated by higher hs-CRP or IL-6 levels, included PA (IL-6 III vs I: OR = 1.78 (95% CI = 1.30; 2.43)), AA (hs-CRP II vs I: 1.18 (1.07; 1.31)), n-6 HUFA (hs-CRP II vs I: 1.16 (1.06; 1.27)), ratio AA/LA (hs-CRP II vs I: 1.38 (1.16; 1.62)) and total SFA (IL-6 III vs I: 2.36 (1.77; 3.15)). FA exposures associated with reduced levels of inflammatory markers included LA (hs-CRP II vs I: 0.90 (0.84; 0.96); IL-6 III vs I: 0.79 (0.69; 0.90)) and total PUFA (IL-6 III vs I: 0.67 (0.57; 0.78)). Sex-stratified sensitivity analyses results are displayed in Supplementary Tables S2a and S2b, for males and females, respectively. As in the total population, both sexes presented a positive association of SFA, and an inverse association of total PUFA with IL-6. Males additionally presented a significant direct association between AA/LA and hs-CRP. Results from the sensitivity analysis using FA concentrations did not differ from those obtained using %FA (data not shown).

DISCUSSION

This exploratory study assessed the associations between FA measured in serum glycerophospholipids and common markers of inflammation (hs-CRP and IL-6) in 10-year-old children. Among our main findings, PA, total SFA, AA, n-6 HUFA and AA/LA were associated with increased low-grade inflammation, as indicated by at least one inflammatory marker. On the other hand, LA and total PUFA were inversely associated with low-grade inflammation.

Few studies exist that describe fatty acid status and markers of inflammation in children, and these differ in terms of study design, methods, location and age of subjects. To aid comparison, an overview of existing studies in both adults and children is presented in Supplementary Table S3. In line with the present findings, González-Gil *et al*.[19] reported increased hs-CRP concentrations with higher AA, n-6 HUFA and AA/LA in a large sample of European children. Given that n-6 HUFA, particularly AA, are known sources of proinflammatory eicosanoids, and that these may increase with greater desaturase activity (estimated by product-to-precursor ratio AA/LA),[31,32] the observed associations with increased levels of inflammation makers are not unexpected. Interestingly, in our study none of the above-mentioned FA exposures presented an association with IL-6, which is the primary CRP regulator.[39,40] This might indicate the involvement of other circulating cytokines. Indeed, interleukin-1β is known to strongly upregulate IL-6-induced CRP production.[41,42] On the other hand, it is possible that differences in hs-CRP were more readily detected given the high sensitivity and stability of this marker, often deeming it first choice for the assessment of low-grade inflammation.[2,5] Although the associations observed with hs-CRP did not indicate a dose–response relationship in the fully adjusted model, the basic model indicated significant associations for n-6 HUFA and AA/LA with both hs-CRP levels II and III relative to level I. By including adjustment variables one by one in the model, it was evident that BMI was the strongest determinant of hs-CRP, as has been observed previously.[19,43]

Following our sex-stratified analysis the association between AA/LA and hs-CRP remained significant only in males. This is in contrast to findings by González-Gil et al.,[19] who reported this association only in females. Previous authors[17] have attributed sex differences to the presence of oestrogen, which enhances the elongation of fatty acids to longer-chain derivatives, such as EPA and DHA,[44,45] which can be anti-inflammatory.[46] Children in the European study were aged 2–9 years,[19] whereas our study was carried out in children aged 10 years, among which about two-thirds of the females had entered onset of puberty. The discrepancy between findings could hence be related to age and in turn hormonal differences.

An association between SFA and low-grade inflammation, as indicated by the present study results, has been previously observed in adults.[16,47] In particular, PA has been shown to induce the expression of IL-6 through the activation of nuclear factor-κ B,[48,49] a protein complex involved in cytokine production. Klein-Platat *et al*.[18] reported a positive association between SFA in plasma phospholipids and IL-6 in overweight adolescents. Like us, the authors observed no association with hs-CRP. Additionally,

Table 1. Descriptive characteristics of study participants

	All, N = *958*	*Males*, N = *520*	*Females*, N = *438*	P-*value*[a]
	n *(%)*	n *(%)*	n *(%)*	
Region				
Munich	487 (50.8)	269 (51.7)	218 (49.8)	0.944
Leipzig	245 (25.6)	130 (25.0)	115 (26.3)	
Bad Honnef	135 (14.1)	72 (13.8)	63 (14.4)	
Wesel	91 (9.5)	49 (9.4)	42 (9.6)	
Mother's education level[b]				
Low	68 (7.1)	39 (7.5)	29 (6.6)	0.455
Medium	339 (35.4)	175 (33.7)	164 (37.4)	
High	551 (57.5)	306 (58.8)	245 (55.9)	
Breast feeding (yes)[c]	927 (96.8)	502 (96.5)	425 (97.0)	0.805
Screen time[d]				
Low	642 (67.0)	327 (62.9)	315 (71.9)	**0.011**
Medium	213 (22.2)	128 (24.6)	85 (19.4)	
High	103 (10.8)	65 (12.5)	38 (8.7)	
Onset of puberty (yes)[e]	429 (44.8)	139 (26.7)	290 (66.2)	< **0.001**
hs-CRP groups[f]				
CRP I	416 (43.4)	265 (51.0)	151 (34.5)	< **0.001**
CRP II	412 (43.0)	198 (38.1)	214 (48.9)	
CRP III	130 (13.6)	57 (11.0)	73 (16.7)	
IL-6 groups[g]				
IL-6 I	751 (78.4)	425 (81.7)	326 (74.4)	**0.019**
IL-6 II	161 (16.8)	72 (13.8)	89 (20.3)	
IL-6 III	46 (4.8)	23 (4.4)	23 (5.3)	
	Mean (s.d.) or median (25th; 75th perc.)	*Mean (s.d.) or median (25th; 75th perc.)*	*Mean (s.d.) or median (25th; 75th perc.)*	
Age (years)	10.2 (10.1; 10.3)	10.2 (10.1; 10.3)	10.2 (10.1; 10.3)	0.900
BMI (kg/m^2)	16.6 (15.5; 18.3)	16.6 (15.6; 18.3)	16.6 (15.4; 18.3)	0.756
hs-CRP (mg/dl)	0.02 (0.01; 0.05)	0.02 (0.01; 0.04)	0.03 (0.02; 0.07)	< **0.001**
IL-6 (pg/ml)	1.5 (1.5; 1.5)	1.5 (1.5; 1.5)	1.5 (1.5; 1.52)	**0.006**
PA (% of total FA)	26.8 (1.1)	26.7 (1.1)	26.9 (1.1)	**0.004**
OA (% of total FA)	12.1 (11.3; 13.1)	12.1 (11.3; 13.1)	12.2 (11.3; 13.1)	0.530
LA (% of total FA)	23.3 (2.3)	23.1 (2.4)	23.4 (2.2)	0.080
GLA (% of total FA)	0.12 (0.09; 0.16)	0.12 (0.1; 0.16)	0.12 (0.09; 0.15)	**0.020**
DHGLA (% of total FA)	3.24 (0.59)	3.29 (0.59)	3.19 (0.6)	**0.007**
AA (% of total FA)	10 (1.6)	10.2 (1.6)	9.83 (1.53)	**0.002**
ALA (% of total FA)	0.24 (0.2; 0.31)	0.25 (0.2; 0.32)	0.24 (0.19; 0.3)	**0.044**
EPA (% of total FA)	0.6 (0.49; 0.75)	0.62 (0.51; 0.77)	0.59 (0.47; 0.72)	**0.008**
DPA (% of total FA)	0.93 (0.8; 1.05)	0.95 (0.81; 1.08)	0.89 (0.78; 1.01)	< **0.001**
DHA (% of total FA)	2.78 (2.29; 3.33)	2.78 (2.33; 3.31)	2.77 (2.28; 3.35)	0.804
n-3 HUFA (% of total FA)	4.37 (3.83; 5.1)	4.42 (3.86; 5.08)	4.32 (3.78; 5.1)	0.162
n-6 HUFA (% of total FA)	14.1 (1.9)	14.3 (1.9)	13.8 (1.8)	< **0.001**
DHA/AA	0.27 (0.24; 0.32)	0.27 (0.24; 0.32)	0.28 (0.24; 0.33)	0.120
EPA/AA	0.06 (0.05; 0.08)	0.06 (0.05; 0.08)	0.06 (0.05; 0.08)	0.359
AA/LA	0.42 (0.37; 0.5)	0.43 (0.37; 0.51)	0.42 (0.37; 0.48)	**0.012**
AA/DHGLA	3.11 (2.65; 3.63)	3.12 (2.68; 3.63)	3.11 (2.63; 3.61)	0.754
n-6/n-3	8.2 (1.76)	8.13 (1.72)	8.29 (1.8)	0.171
SFA (% of total FA)	42.2 (1.2)	42.1 (1.3)	42.4 (1.1)	**0.003**
MUFA (% of total FA)	14.4 (13.5; 15.3)	14.3 (13.5; 15.3)	14.5 (13.6; 15.4)	0.183
PUFA (% of total FA)	42.8 (2.2)	43 (2.2)	42.6 (2.1)	**0.017**

Abbreviations: AA, arachidonic acid; ALA, α-linoleic acid; BMI, body mass index; DHA, docosahexaenoic acid; DHGLA, dihomo-γ-linoleic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; hs-CRP, high-sensitivity C-reactive protein; HUFA, highly unsaturated FA; IL, interelukin; LA, linoleic acid; MUFA, docosahexaenoic acid; OA, oleic acid; PA, palmitic acid; PUFA, polyunsaturated FA; SFA, saturated FA. Values are presented as counts (%) for categorical variables, mean (s.d.) for normally distributed continuous variables and median (25th; 75th percentile) for non-normally distributed continuous variables. [a]Comparison between males and females: tested by Student's *t*-test (means) or by Wilcoxon's rank-sum test (medians) for continuous variables, and by Pearson's χ^2 test for categorical variables. Significant *P*-values are marked in bold ($P < 0.05$). [b]Highest level achieved (low: < 10th grade; medium: 10th grade; high: > 10th grade). [c]Whether the child was ever breastfed (yes: ⩾ 1 month). [d]Self-reported h per day spent on screen activities (low: ⩽ 1 h in winter and ⩽ 2 h in summer; medium: > 1 h in winter or > 2 h in summer; high: > 1 h in winter and > 2 h in summer). [e]Females: estradiol > 18.4 pmol/l; males: testosterone > 0.09 nmol/l. [f](I) hs-CRP < 0.02 mg/dl; (II) hs-CRP ⩾ 0.02 mg/dl and < 75th sex-specific percentile of those with hs-CRP ⩾ 0.02 mg/dl (< 0.11 mg/dl in girls; < 0.09 mg/dl in boys); and (III) hs-CRP ⩾ 75th sex-specific percentile of those with hs-CRP ⩾ 0.02 mg/dl (⩾0.11 mg/dl in girls; ⩾ 0.09 mg/dl in boys). [g](I) IL-6 ⩽ 1.5 pg/ml; (II) IL-6 > 1.5 pg/ml and < 75th sex-specific percentile of those with IL-6 > 1.5 pg/ml (< 4.26 pg/ml in girls; < 3.93 pg/ml in boys); and (III) IL-6 ⩾75th sex-specific percentile of those with IL-6 > 1.5 pg/ml (⩾4.26 pg/ml in girls; ⩾ 3.93 pg/ml in boys).

Table 2. OR and 95% CI assessing the association of fatty acid exposures with hs-CRP and IL-6 categories

Fatty acids	*hs-CRP category II* vs *I*			*hs-CRP category III* vs *I*			*IL-6 category II* vs *I*			*IL-6 category III* vs *I*		
	OR	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*
PA (%FA)												
M1	1.04	0.91; 1.20	0.560	1.33	1.09; 1.62	0.006	1.07	0.90; 1.27	0.460	1.74	1.28; 2.36	< **0.001**
M2	0.99	0.86; 1.15	0.935	1.24	1.00; 1.53	0.051	1.03	0.86; 1.23	0.728	1.78	1.30; 2.43	< **0.001**
OA (%FA)												
M1	0.92	0.83; 1.03	0.148	0.91	0.78; 1.07	0.257	1.05	0.92; 1.21	0.481	1.38	1.10; 1.73	0.006
M2	0.97	0.86; 1.09	0.599	0.98	0.83; 1.16	0.837	1.08	0.94; 1.24	0.293	1.35	1.08; 1.71	0.010
LA (%FA)												
M1	0.85	0.80; 0.91	< **0.001**	0.82	0.75; 0.90	< **0.001**	0.95	0.88; 1.03	0.207	0.82	0.72; 0.94	0.003
M2	0.90	0.84; 0.96	**0.001**	0.90	0.82; 0.99	0.030	0.99	0.91; 1.07	0.709	0.79	0.69; 0.90	< **0.001**
GLA[a] *(%FA)*												
M1	1.22	0.96; 1.55	0.102	1.22	0.87; 1.73	0.254	1.11	0.82; 1.49	0.506	0.98	0.58; 1.66	0.947
M2	1.06	0.82; 1.37	0.642	0.97	0.67; 1.41	0.875	1.04	0.77; 1.41	0.811	1.04	0.61; 1.76	0.899
DHGLA (%FA)												
M1	1.35	1.06; 1.73	0.016	1.47	1.04; 2.09	0.029	1.16	0.87; 1.56	0.322	1.16	0.69; 1.93	0.577
M2	1.12	0.87; 1.45	0.386	1.07	0.73; 1.55	0.731	1.05	0.78; 1.43	0.747	1.27	0.75; 2.13	0.376
AA (%FA)												
M1	1.24	1.12; 1.37	< **0.001**	1.22	1.05; 1.40	0.008	0.96	0.85; 1.09	0.558	0.92	0.75; 1.14	0.460
M2	1.18	1.07; 1.31	**0.002**	1.13	0.97; 1.32	0.111	0.93	0.83; 1.06	0.281	0.94	0.76; 1.17	0.605
ALA[a] *(%FA)*												
M1	0.95	0.82; 1.10	0.488	0.83	0.66; 1.05	0.121	1.02	0.85; 1.23	0.834	1.12	0.83; 1.52	0.447
M2	0.92	0.79; 1.08	0.291	0.80	0.63; 1.02	0.068	1.01	0.84; 1.23	0.909	1.12	0.83; 1.52	0.455
EPA (%FA)												
M1	2.12	1.23; 3.65	0.007	1.77	0.81; 3.88	0.151	1.80	0.99; 3.29	0.055	0.45	0.11; 1.78	0.253
M2	1.40	0.80; 2.47	0.239	0.90	0.38; 2.13	0.816	1.53	0.82; 2.87	0.182	0.53	0.13; 2.12	0.368
DPA (%FA)												
M1	1.53	0.65; 3.62	0.333	0.51	0.14; 1.84	0.302	0.60	0.20; 1.78	0.354	0.19	0.03; 1.32	0.093
M2	1.33	0.53; 3.29	0.545	0.34	0.09; 1.31	0.116	0.53	0.17; 1.62	0.264	0.20	0.03; 1.41	0.106
DHA (%FA)												
M1	1.15	0.95; 1.38	0.154	1.11	0.85; 1.46	0.437	0.93	0.73; 1.18	0.536	0.62	0.39; 0.98	0.039
M2	1.13	0.92; 1.37	0.240	1.05	0.79; 1.41	0.733	0.90	0.70; 1.14	0.375	0.63	0.40; 1.01	0.054
n-3 HUFA (%FA)												
M1	1.16	1.00; 1.34	0.044	1.09	0.883; 1.344	0.422	0.99	0.83; 1.18	0.918	0.67	0.47; 0.96	0.031
M2	1.11	0.95; 1.29	0.180	0.99	0.79; 1.241	0.930	0.95	0.79; 1.15	0.609	0.69	0.48; 1.00	0.047
n-6 HUFA (%FA)												
M1	1.23	1.13; 1.35	< **0.001**	1.23	1.08; 1.39	**0.002**	0.99	0.88; 1.10	0.799	0.97	0.80; 1.17	0.735
M2	1.16	1.06; 1.27	**0.002**	1.11	0.97; 1.27	0.134	0.95	0.85; 1.06	0.344	1.00	0.82; 1.21	0.978
DHA/AA[a]												
M1	0.90	0.74; 1.09	0.281	0.89	0.67; 1.18	0.430	0.95	0.75; 1.21	0.688	0.63	0.39; 1.03	0.067
M2	0.93	0.76; 1.14	0.458	0.91	0.67; 1.22	0.528	0.95	0.74; 1.22	0.684	0.63	0.39; 1.04	0.069
EPA/AA[a]												
M1	1.37	0.82; 2.29	0.232	1.34	0.64; 2.80	0.440	1.75	0.98; 3.13	0.061	0.54	0.15; 2.01	0.359
M2	1.01	0.59; 1.73	0.973	0.83	0.37; 1.84	0.638	1.56	0.85; 2.84	0.149	0.61	0.16; 2.25	0.455
AA/LA[a]												
M1	1.54	1.31; 1.80	< **0.001**	1.58	1.27; 1.98	< **0.001**	1.00	0.83; 1.21	0.998	1.19	0.88; 1.62	0.263
M2	1.38	1.16; 1.62	< **0.001**	1.31	1.04; 1.67	0.023	0.92	0.76; 1.12	0.426	1.28	0.93; 1.76	0.129
AA/DHGLA[a]												
M1	1.01	0.99; 1.03	0.483	1.01	0.98; 1.03	0.588	0.99	0.97; 1.01	0.283	0.98	0.94; 1.03	0.461
M2	1.01	0.99; 1.03	0.204	1.02	0.99; 1.05	0.195	0.99	0.97; 1.01	0.391	0.98	0.94; 1.03	0.404
n-6/n-3[a]												
M1	0.99	0.98; 1.00	0.005	0.99	0.98; 1.00	0.167	1.00	0.99; 1.01	0.883	1.01	0.99; 1.02	0.493
M2	0.99	0.98; 1.00	0.039	1.00	0.99; 1.01	0.710	1.00	0.99; 1.01	0.722	1.00	0.99; 1.02	0.639

Table 2. (Continued)

Fatty acids	*hs-CRP category II* vs *I*			*hs-CRP category III* vs *I*			*IL-6 category II* vs *I*			*IL-6 category III* vs *I*		
	OR	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*	*OR*	*95% CI*	P-*value*
SFA (% of total FA)												
M1	1.20	1.04; 1.38	0.012	1.53	1.26; 1.85	< **0.001**	1.19	1.00; 1.42	0.046	2.17	1.65; 2.86	< **0.001**
M2	1.08	0.93; 1.25	0.312	1.32	1.08; 1.63	0.008	1.13	0.94; 1.35	0.187	2.36	1.77; 3.15	< **0.001**
MUFA (% of total FA)												
M1	0.97	0.87; 1.07	0.516	0.97	0.84; 1.13	0.711	1.06	0.93; 1.21	0.362	1.32	1.07; 1.63	0.010
M2	0.99	0.89; 1.10	0.875	1.01	0.86; 1.18	0.931	1.07	0.94; 1.22	0.291	1.31	1.05; 1.62	0.015
PUFA (% of total FA)												
M1	0.97	0.90; 1.04	0.369	0.90	0.81; 1.00	0.040	0.92	0.83; 1.01	0.067	0.68	0.58; 0.79	< **0.001**
M2	0.98	0.91; 1.06	0.657	0.92	0.82; 1.03	0.146	0.93	0.84; 1.02	0.118	0.67	0.57; 0.78	< **0.001**

Abbreviations: AA, arachidonic acid; ALA, α-linoleic acid; BMI, body mass index; CI, confidence interval; DHA, docosahexaenoic acid; DHGLA, dihomo-γ-linoleic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; hs-CRP, high-sensitivity C-reactive protein; HUFA, highly unsaturated FA; IL, interelukin; LA, linoleic acid; MUFA, docosahexaenoic acid; OA, oleic acid; OR, odds ratio; PA, palmitic acid; PUFA, polyunsaturated FA; SFA, saturated FA. Multinomial logistic regression models adjusting for: (M1) sex, region, age and maternal education level; and (M2) further adjusting for BMI, screen time, onset of puberty and whether the child was ever breastfed. [a]OR for these variables refer to changes of 0.1 U. Significant associations are marked in bold (Bonferroni-corrected *P*-value < 0.0025).

neither PA nor total SFA showed significant associations with hs-CRP in the recent European study population.[19] To our knowledge, the present findings are the first to indicate a role of SFA, namely PA, in triggering proinflammatory responses in otherwise healthy children, irrespective of BMI.

We observed inverse associations with low-grade inflammation for LA, the main dietary n-6 PUFA, and for total PUFA. Although the anti-inflammatory role of n-3 PUFA has been more extensively investigated, a number of studies in both adults and children have reported reduced concentrations of inflammatory markers with higher total n-6 PUFA levels,[14,16,17] and specifically with LA.[15,19,50] There is some evidence suggesting that the presence of double bonds, regardless of the position of the bond (n-3 or n-6), may have a relevant role in reducing inflammation.[51] In contrast to these findings, it has been argued that high LA consumption could induce inflammation through its endogenous conversion to AA, which can act as substrate for synthesis of proinflammatory molecules.[26,52] However, little evidence currently supports a proinflammatory role of LA in humans.[28,53] It has been shown that AA production from LA is tightly regulated,[54] and tissue AA content is barely altered by LA intake,[55] even in the context of a high n-6/n-3 ratio.[56] Furthermore, LA and AA are known to produce both proinflammatory and resolving metabolites and could therefore contribute to anti-inflammatory responses as well.[9] Our results do not support the theoretical role of n-6/n-3 in modulating inflammation, proposed on the basis that LA can diminish the conversion of ALA to EPA.[26] Although true to some extent,[57] the conversion of dietary n-3 PUFA to long-chain derivatives in humans is low,[58] and small changes are likely not highly relevant in terms of the overall inflammatory process. Furthermore, achieving a lower n-6/n-3 ratio by limiting intakes of n-6 PUFA has not consistently resulted in improved cardiovascular risk.[59] In this context, and in line with existing literature, our findings suggest that elevated LA in serum phospholipids, within the ranges observed in the current study, is not detrimental in terms of inflammatory processes in children; rather, both LA and total PUFA seem to promote an anti-inflammatory response.[60]

Strengths and limitations

The present study adds to the limited literature on associations of FA composition with markers of inflammation in children, and benefits from a large, homogenous study population. A main strength in our study is the analysis of FA status, reflecting individual dietary FA intake and endogenous metabolism. FA measured in serum phospholipids have been shown to reflect FA intake over a period of weeks to months,[60] making them acceptable markers of habitual FA intake. Among the different lipid fractions, phospholipids contain the highest percentages of DHA and AA, hence allowing a more precise analysis of FA composition.[61] Although sample alterations during handling and storage cannot be completely excluded, serum samples obtained in our study were frozen directly after sampling and stored at –80 °C until analysis. Furthermore, time until centrifugation was short and haemolysis was minimal, thereby limiting the probability of exchange of phospholipids between cells and serum.

Our findings are based on the analysis of percentage of FA relative to total FA (%FA). Despite its use in most studies, this method is limited by the inability to account for actual FA concentrations.[62] However, additional analyses in our study sample indicated similar results for both methods. Given the exploratory nature of the current study, a large number of FA exposures were assessed. The multiple tests and possible correlation between FA exposures, increases the probability of occurrence of chance findings. We therefore applied a rather conservative approach to correct for multiple testing. Observed significant associations were in line with existing literature and the directions of associations for both inflammatory outcomes were generally consistent, suggesting that our findings are unlikely to have arisen by chance. Furthermore, we are aware that the AA/LA ratio assessed in our study represents an indirect measurement of desaturase activity. However, it has been shown that single-nucleotide polymorphisms and haplotypes of the genes coding for desaturase enzymes are associated with relative proportions of serum phospholipid FA,[63] and the use of the AA/LA ratio as a marker of overall desaturase activity is widespread.[31,32,64]

As often occurring in cohort studies, children of lower social classes were underrepresented in the present analyses. Although we adjusted for parental education in our analysis, our findings may not be representative of the study area. Additional assessment of other cytokine measurements, which unfortunately were not available from the LISAplus cohort, would have been useful to strengthen our conclusions and better understand the possible inflammatory pathways involved. Finally, it must be kept

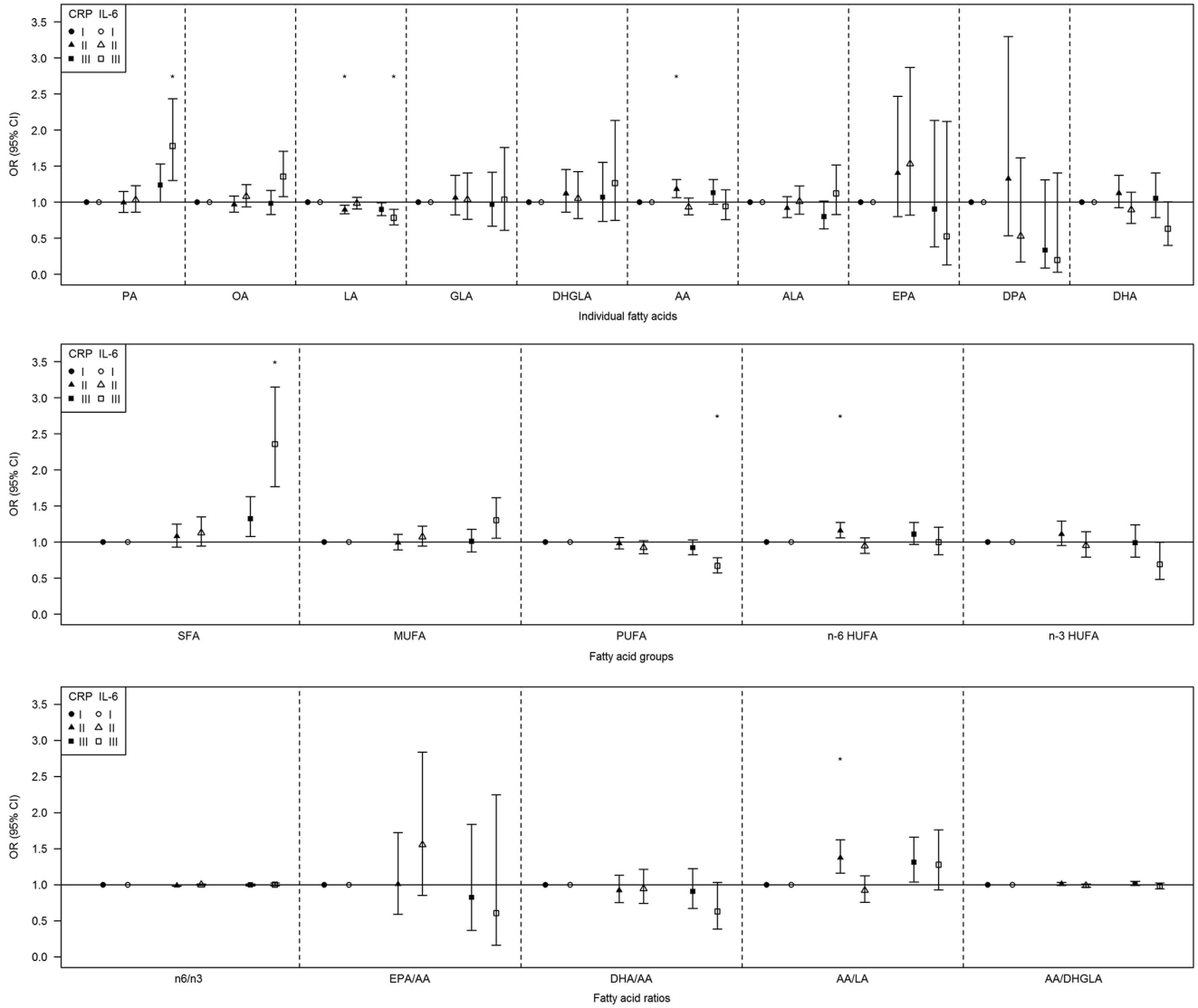

Figure 2. OR and 95% CI of the associations between fatty acids and categories of hs-CRP and IL-6 (reference: category I). Multinomial logistic regression model (M2) adjusted for sex, region, age, maternal education level, BMI, sedentary behaviour and whether the child was ever breastfed. Significant associations are marked with an asterisk.

in mind that our findings are based on cross-sectional analyses, and hence the observed associations between serum FA and inflammatory markers do not necessarily infer causality.

CONCLUSION

The results of this exploratory study suggest that higher SFA and n-6 HUFA, namely PA and AA, are associated with increased levels of low-grade inflammation in children, as indicated by the inflammatory markers IL-6 and hs-CRP. In contrast, the major dietary n-6 PUFA and total PUFA are associated with reduced levels of low-grade inflammation. Elevated desaturase activity, estimated by the ratio AA/LA, may be associated with increased inflammation, particularly in boys. Sex might play a relevant role in the underlying inflammatory mechanisms in children, and should be kept in mind for future studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank all the families for their participation in the LISAplus studies. Furthermore, we thank all members of the LISAplus study groups for their excellent work.The LISAplus study was mainly supported by grants from the Federal Ministry for Education, Science, Research and Technology and in addition from Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research—UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Paediatric Practice, Bad Honnef for the first 2 years. The 4-, 6- and 10-year follow-up examinations of the LISAplus study were covered from the respective budgets of the involved partners (Helmholtz Zentrum Munich (former GSF), Helmholtz Centre for Environmental Research—UFZ, Leipzig, Research Institute at Marien-Hospital Wesel, Paediatric Practice, Bad Honnef, IUF—Leibniz-Research Institute for Environmental Medicine at the University of Düsseldorf) and in addition by a grant from the Federal Ministry for Environment (IUF Düsseldorf, FKZ 20462296). The work of BK is financially supported in part by the European Research Council Advanced Grant META-GROWTH (ERC-2012-AdG—no.322605).

MEMBERS OF THE LISAPLUS STUDY GROUP

The LISAplus Study group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Munich (J Heinrich, M Schnappinger,

I Brüske, M Sußmann, E Thiering, C Tiesler, C Flexeder, H Schulz, C Zeller, M Standl); Department of Pediatrics, Municipal Hospital 'St Georg', Leipzig (M Borte, U Diez, C Dorn, E Braun); Marien-Hospital Wesel, Department of Pediatrics, Wesel (A von Berg); Paediatric Practice, Bad Honnef (B Schaaf); Helmholtz Centre of Environmental Research—UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (I Lehmann, M Bauer, S Röder, M Schilde, G Herberth, J Müller); Technical University Munich, Department of Pediatrics, Munich (U Hoffmann, CP Bauer); Technical University Munich, ZAUM—Center for Allergy and Environment, Munich (M Ollert, J Grosch).

AUTHOR CONTRIBUTIONS

CH, JH and MS were involved in the conception and design of the study; BK, HD, IL, AvB and JH in the data acquisition; CH, MS and CF in the statistical analyses; CH, MS, HD and JH in the interpretation; CH drafted the manuscript; all authors revised it critically for important intellectual content, and approved the final version to be published.

REFERENCES

1 Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2045–2051.
2 Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. *Am J Med* 2004; **116**: 9s–16s.
3 Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006; **6**: 772–783.
4 Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999; **282**: 2131–2135.
5 Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003; **111**: 1805–1812.
6 Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J *et al.* Elevated serum C-reactive protein levels and early arterial changes in healthy children. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1323–1328.
7 Kapiotis S, Holzer G, Schaller G, Haumer M, Widhalm H, Weghuber D *et al.* A proinflammatory state is detectable in obese children and is accompanied by functional and morphological vascular changes. *Arterioscler Thromb Vasc Biol* 2006; **26**: 2541–2546.
8 Calder PC, Albers R, Antoine JM, Blum S, Bourdet-Sicard R, Ferns GA *et al.* Inflammatory disease processes and interactions with nutrition. *Br J Nutr* 2009; **101** (Suppl 1): S1–45.
9 Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie* 2009; **91**: 791–795.
10 Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol* 2013; **75**: 645–662.
11 Fekete K, Marosvölgyi T, Jakobik V, Decsi T. Methods of assessment of n-3 long-chain polyunsaturated fatty acid status in humans: a systematic review. *Am J Clin Nutr* 2009; **89**: 2070S–2084SS.
12 Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. *Eur J Clin Nutr* 2000; **54**: 367–372.
13 Nakamura MT, Nara TY. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annu Rev Nutr* 2004; **24**: 345–376.
14 Ferrucci L, Cherubini A, Bandinelli S, Bartali B, Corsi A, Lauretani F *et al.* Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* 2006; **91**: 439–446.
15 Steffen BT, Steffen LM, Tracy R, Siscovick D, Hanson NQ, Nettleton J *et al.* Obesity modifies the association between plasma phospholipid polyunsaturated fatty acids and markers of inflammation: the Multi-Ethnic Study of Atherosclerosis. *Int J Obes (Lond)* 2012; **36**: 797–804.
16 Kaikkonen JE, Kresanov P, Ahotupa M, Jula A, Mikkila V, Viikari JS *et al.* High serum n6 fatty acid proportion is associated with lowered LDL oxidation and inflammation: the Cardiovascular Risk in Young Finns Study. *Free Radic Res* 2014; **48**: 420–426.
17 Muka T, Kiefte-de Jong JC, Hofman A, Dehghan A, Rivadeneira F, Franco OH. Polyunsaturated fatty acids and serum C-reactive protein: the Rotterdam study. *Am J Epidemiol* 2015; **181**: 846–856.
18 Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. *Am J Clin Nutr* 2005; **82**: 1178–1184.
19 González-Gil EM, Santabárbara J, Siani A, Ahrens W, Sioen I, Eiben G *et al.* Whole-blood fatty acids and inflammation in European children: the IDEFICS Study. *Eur J Clin Nutr* 2016; **70**: 819–823.
20 Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I, Fahlbusch B *et al.* Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *Eur Respir J* 2002; **20**: 617–623.
21 Glaser C, Rzehak P, Demmelmair H, Klopp N, Heinrich J, Koletzko B *et al.* Influence of FADS polymorphisms on tracking of serum glycerophospholipid fatty acid concentrations and percentage composition in children. *PLoS One* 2011; **6**: e21933.
22 Glaser C, Demmelmair H, Sausenthaler S, Herbarth O, Heinrich J, Koletzko B. Fatty acid composition of serum glycerophospholipids in children. *J Pediatr* 2010; **157**: 826–831. e1.
23 Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich J. Age-dependent effects of cord blood long-chain PUFA composition on BMI during the first 10 years of life. *Br J Nutr* 2014; **111**: 2024–2031.
24 Glaser C, Demmelmair H, Koletzko B. High-throughput analysis of fatty acid composition of plasma glycerophospholipids. *J Lipid Res* 2010; **51**: 216–221.
25 Lands B. Consequences of essential fatty acids. *Nutrients* 2012; **4**: 1338–1357.
26 Simopoulos AP. The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp Biol Med (Maywood)* 2008; **233**: 674–688.
27 Marventano S, Kolacz P, Castellano S, Galvano F, Buscemi S, Mistretta A *et al.* A review of recent evidence in human studies of n-3 and n-6 PUFA intake on cardiovascular disease, cancer, and depressive disorders: does the ratio really matter? *Int J Food Sci Nutr* 2015; **66**: 611–622.
28 Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ *et al.* Omega-6 fatty acids and risk for cardiovascular disease. A Science Advisory From the American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation* 2009; **119**: 902–907.
29 Ohnishi H, Saito Y. Eicosapentaenoic acid (EPA) reduces cardiovascular events: relationship with the EPA/arachidonic acid ratio. *J Atheroscler Thromb* 2013; **20**: 861–877.
30 Cotogni P, Trombetta A, Muzio G, Maggiora M, Canuto RA. The omega-3 fatty acid docosahexaenoic acid modulates inflammatory mediator release in human alveolar cells exposed to bronchoalveolar lavage fluid of ARDS patients. *Biomed Res Int* 2015; **2015**: 642520.
31 Martinelli N, Girelli D, Malerba G, Guarini P, Illig T, Trabetti E *et al.* FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. *Am J Clin Nutr* 2008; **88**: 941–949.
32 Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of fatty acids and insulin action. *Ann NY Acad Sci* 2002; **967**: 183–195.
33 R Core Team. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing*, Vienna, Austria, 2016.
34 Venables WN, Ripley BD. *Modern Applied Statistics with S*, 4th edn. Springer: New York, NY, USA, 2002.
35 Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta* 1981; **117**: 13–23.
36 Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine levels in the serum of healthy subjects. *Mediat Inflamm* 2013; **2013**: 6.
37 Vittinghoff E, McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol* 2007; **165**: 710–718.
38 Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. *J Clin Epidemiol* 1996; **49**: 1373–1379.
39 Black S, Kushner I, Samols D. C-reactive protein. *J Biol Chem* 2004; **279**: 48487–48490.
40 Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R *et al.* Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 1989; **242**: 237–239.
41 Bode JG, Albrecht U, Häussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins—regulation by IL-6-and IL-1-type cytokines involving STAT3 and its crosstalk with NF-κB-dependent signaling. *Eur J Cell Biol* 2012; **91**: 496–505.
42 Ganter U, Arcone R, Toniatti C, Morrone G, Ciliberto G. Dual control of C-reactive protein gene expression by interleukin-1 and interleukin-6. *EMBO J* 1989; **8**: 3773–3779.
43 Ford ES. C-reactive protein concentration and cardiovascular disease risk factors in children: findings from the National Health and Nutrition Examination Survey 1999–2000. *Circulation* 2003; **108**: 1053–1058.
44 Decsi T, Kennedy K. Sex-specific differences in essential fatty acid metabolism. *Am J Clin Nutr* 2011; **94** (Suppl): 1914S–1919SS.

45 Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr* 2002; **88**: 411–420.
46 James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr* 2000; **71** (Suppl): 343s–348s.
47 King DE, Egan BM, Geesey ME. Relation of dietary fat and fiber to elevation of C-reactive protein. *Am J Cardiol* 2003; **92**: 1335–1339.
48 Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F, Haring HU *et al.* Palmitate, but not unsaturated fatty acids, induces the expression of interleukin-6 in human myotubes through proteasome-dependent activation of nuclear factor--kappaB. *J Biol Chem* 2004; **279**: 23942–23952.
49 Ajuwon KM, Spurlock ME. Palmitate activates the NF-κB transcription factor and induces IL-6 and TNFα expression in 3T3-L1 adipocytes. *J Nutr* 2005; **135**: 1841–1846.
50 Enzenbach C, Kroger J, Zietemann V, Jansen EH, Fritsche A, Doring F *et al.* Erythrocyte membrane phospholipid polyunsaturated fatty acids are related to plasma C-reactive protein and adiponectin in middle-aged German women and men. *Eur J Nutr* 2011; **50**: 625–636.
51 De Caterina R, Liao JK, Libby P. Fatty acid modulation of endothelial activation. *Am J Clin Nutr* 2000; **71** (Suppl): 213s–223ss.
52 Lands B. Historical perspectives on the impact of n-3 and n-6 nutrients on health. *Prog Lipid Res* 2014; **55**: 17–29.
53 Johnson GH, Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. *J Acad Nutr Diet* 2012; **112**: 1029–1041, 41.e1–41.e15.
54 Mohrhauer H, Holman RT. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *J Lipid Res* 1963; **4**: 151–159.
55 Rett BS, Whelan J. Increasing dietary linoleic acid does not increase tissue arachidonic acid content in adults consuming Western-type diets: a systematic review. *Nutr Metab (Lond)* 2011; **8**: 36.
56 Bjermo H, Iggman D, Kullberg J, Dahlman I, Johansson L, Persson L *et al.* Effects of n-6 PUFAs compared with SFAs on liver fat, lipoproteins, and inflammation in abdominal obesity: a randomized controlled trial. *Am J Clin Nutr* 2012; **95**: 1003–1012.
57 Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J Nutr* 2007; **137**: 945–952.
58 Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr Opin Clin Nutr Metab Care* 2002; **5**: 127–132.
59 FAO. *Fats and fatty acids in human nutrition. Report of an expert consultation*. FAO food and nutrition paper no. 19, 2010, pp 1–166.
60 Riboli E, Ronnholm H, Saracci R. Biological markers of diet. *Cancer Surv* 1987; **6**: 685–718.
61 Rise P, Eligini S, Ghezzi S, Colli S, Galli C. Fatty acid composition of plasma, blood cells and whole blood: relevance for the assessment of the fatty acid status in humans. *Prostaglandins Leukot Essent Fatty Acids* 2007; **76**: 363–369.
62 Sergeant S, Ruczinski I, Ivester P, Lee TC, Morgan TM, Nicklas BJ *et al.* Impact of methods used to express levels of circulating fatty acids on the degree and direction of associations with blood lipids in humans. *Br J Nutr* 2016; **115**: 251–261.
63 Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I *et al.* Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum Mol Genet* 2006; **15**: 1745–1756.
64 Steffen LM, Vessby B, Jacobs DR Jr, Steinberger J, Moran A, Hong CP *et al.* Serum phospholipid and cholesteryl ester fatty acids and estimated desaturase activities are related to overweight and cardiovascular risk factors in adolescents. *Int J Obes (Lond)* 2008; **32**: 1297–1304.

Supplementary Information accompanies this paper on European Journal of Clinical Nutrition website (http://www.nature.com/ejcn)