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

Carla Harris[1], Hans Demmelmair[2], Andrea von Berg[3], Irina Lehmann[4,5], Claudia Flexeder[1], Berthold Koletzko[2], Joachim Heinrich[1,6], Marie Standl[1*]

[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] Research Institute, Department of Pediatrics, 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

[6] Institute and Outpatient Clinic for Occupational, Social and Environmental Medicine, Inner City Clinic, University Hospital of Munich (LMU), Munich, Germany

* Corresponding Author:

Dr. Marie Standl

Helmholtz Zentrum München

Institute of Epidemiology I

Ingolstädter Landstr. 1

85764 Neuherberg

Germany

E-Mail: marie.standl@helmholtz-muenchen.de

Tel.: +498931872952

Fax: +498931873380

Running title:

Fatty acids and low-grade inflammation in children

Conflict of interest:

The authors declare no conflict of interest.

Abstract

Background: 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.

Methods: Complete data were available for 958 participants from the 10-year follow-up of the LISAplus birth cohort study. FA composition was assessed in serum glycerophospholipids. Hs-CRP and IL-6 were categorized into 3 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: linoleic acid (LA) (hs.CRP: $p=0.001$, 0.84;0.96, IL-6: $p<0.001$, 0.69;0.90) and total polyunsaturated FA (PUFA) ($p<0.001$, 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.

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 (FA), 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 levels of arachidonic acid (AA), a known source of pro-inflammatory 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 play relevant roles in inflammatory processes, with common markers of inflammation in 10-year-old children, namely high-sensitivity CRP (hs-CRP) and IL-6.

Methods

Data was obtained from the 10-year follow-up assessment of the ongoing LISAplus (Influence of *L*ifestyle-Related Factors on the *I*mmune *S*ystem and the Development of *A*llergies 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 new-borns 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 3000U/min for 10 minutes 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 by flow cytometry using a cytometric bead array (BD CBA Human Soluble Flex Set system; Becton Dickinson, Heidelberg, Germany), according to 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 categorized into 3 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 < 0.02mg/dl; (II) hs-CRP ≥ 0.02mg/dl and < 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (< 0.11mg/dl in girls; < 0.09mg/dl in boys); and (III) hs-CRP ≥ 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (≥ 0.11mg/dl in girls; ≥ 0.09mg/dl in boys). IL-6 was categorized with reference to the minimal detectable concentration (1.5pg/ml): (I) IL-6 ≤ 1.5pg/ml; (II) IL-6 > 1.5pg/ml and < 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (< 4.26pg/ml in girls; < 3.93pg/ml in

boys); and (III) IL-6 ≥ 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (≥ 4.26pg/ml in girls; ≥ 3.93pg/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), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), total SFA, total monounsaturated FA (MUFA) and total PUFA. Additionally, we included FA groups and ratios which have previously been proposed to play a role in inflammation. Highly unsaturated n-6 and n-3 FA (HUFA: ≥20 carbons and ≥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 (n6/n3) 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 n6/n3 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 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.

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: $<10^{th}$grade; medium: 10^{th}grade; high: $>10^{th}$grade), BMI (in kg/m^2, calculated from height and weight measurements obtained at the physical examination), screen time (low: ≤1h in winter and ≤2h in summer; medium: >1h in winter or >2h in summer; high: >1h in winter and >2h in summer), onset of puberty (yes: estradiol >18.4pmol/L in females; testosterone >0.09nmol/L in males), and whether the child was ever breastfed (yes: ≥1 month).

206

207 *Statistical analysis*

208 Differences in characteristics between girls and boys were tested by
209 Student's t-test (means) or by Wilcoxon rank sum test (medians) for
210 continues variables, and by Pearson's Chi-squared test for categorical
211 variables. A two-sided α-level of 5% was considered significant.
212 Associations of %FA with hs-CRP and IL-6 were assessed using
213 multinomial logistic regression, given that the outcome variables hs-
214 CRP and IL-6 were both categorised into 3 levels (ordinal logistic
215 regression could not be applied, as the assumption of proportional
216 odds was not satisfied). Results were presented as odds ratios with
217 corresponding 95% confidence intervals [OR (95% CI)], with the
218 lowest level (I) as the reference category. A basic model (M1) and a
219 fully adjusted model (M2) were used, adjusting for: (M1) sex, region,
220 age and maternal education level; and (M2) further adjusting for
221 BMI, screen time, onset of puberty and whether the child was ever
222 breastfed. Sensitivity analyses were run stratified by sex. In order to
223 avoid chance findings resulting from the large number of regression
224 models, we corrected for multiple testing using Bonferroni
225 correction: the alpha level was divided by twenty (the number of tests
226 performed). This yielded a corrected two-sided α-level of 0.0025
227 (0.05/20 = 0.0025). For sex-stratified analyses, the p-value was
228 further divided by two, accounting for the analysis at two levels
229 (0.0025/2=0.00125). Finally, we reran our analyses using FA
230 concentrations, including adjustment for total FA. To avoid problems
231 of multicollinearity, we used FA residuals calculated by regressing
232 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 > 1mg/dl (35) or IL-6 values > 20pg/ml (36) were considered as outliers and excluded from the analysis (7 subjects with hs-CRP levels from 1.03-4.37mg/dl and 6 subjects with IL-6 levels from 32.9-4384.0pg/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 to just 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. Amongst 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 which describe fatty acid status and markers of inflammation in children, and these differ in terms of study design, methods, location, and age of subjects. In order 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. reported increased hs-CRP concentrations with higher AA, n-6 HUFA, and AA/LA in a large sample of European children (19). Given that n-6 HUFA, particularly AA, are known sources of pro-inflammatory 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 up-regulate 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., who reported this association only in females (19). 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 10-year-olds, 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 activation of nuclear factor-kB (48, 49), a protein complex involved in cytokine production. Klein Platat et al. reported a positive association between SFA in plasma phospholipids and IL-6 in overweight adolescents (18). Like us, the authors observed no association with hs-CRP. Additionally, neither palmitic acid 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 pro-inflammatory 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 play 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 n6/n3 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 n6/n3 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 n6/n3 ratio by limiting intakes of n-6 PUFA has not consistently resulted in improved CVD 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,

366 both LA and total PUFA seem to promote an anti-inflammatory
367 response (60).
368
369 *Strengths and limitations*
370 The present study adds to the limited literature on associations of FA
371 composition with markers of inflammation in children, and benefits
372 from a large, homogenous study population. A main strength in our
373 study is the analysis of FA status, reflecting individual dietary FA
374 intake and endogenous metabolism. FA measured in serum
375 phospholipids have been shown to reflect FA intake over a period of
376 weeks to months (60), making them acceptable markers of habitual
377 FA intake. Among the different lipid fractions, phospholipids
378 contain the highest percentages of DHA and AA, hence allowing a
379 more precise analysis of FA composition (61). Although sample
380 alterations during handling and storage cannot be completely
381 excluded, serum samples obtained in our study were frozen directly
382 after sampling and stored at –80°C until analysis. Furthermore, time
383 until centrifugation was short and haemolysis was minimal, thereby
384 limiting the probability of exchange of phospholipids between cells
385 and serum.
386 Our findings are based on the analysis of percentage of FA relative to
387 total FA (%FA). Despite its use in most studies, this method is
388 limited by the inability to account for actual FA concentrations (62).
389 However, additional analyses in our study sample indicated similar
390 results for both methods. Given the exploratory nature of the current
391 study, a large number of FA exposures were assessed. The multiple
392 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 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 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.

Authorship

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.

Acknowledgements

The authors 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 group consists of the following: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology I, Munich (Heinrich J, Schnappinger M, Brüske I, Sußmann M, Lohr W, Schulz H, Zeller C, Standl M); Department of Pediatrics, Municipal Hospital "St. Georg", Leipzig (Borte M, Gnodtke E); Marien Hospital Wesel,

Department of Pediatrics, Wesel (von Berg A, Berdel D, Stiers G, Maas B); Pediatric Practice, Bad Honnef (Schaaf B); Helmholtz Centre of Environmental Research – UFZ, Department of Environmental Immunology/Core Facility Studies, Leipzig (Lehmann I, Bauer M, Röder S, Schilde M, Nowak M, Herberth G , Müller J, Hain A); Technical University Munich, Department of Pediatrics, Munich (Hoffmann U, Paschke M, Marra S); Clinical Research Group Molecular Dermatology, Department of Dermatology and Allergy, Technische Universität München (TUM), Munich (Ollert M).

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, Pediatric Practice, Bad Honnef for the first 2 years. The 4 year, 6 year, 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, Pediatric 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).

Conflict of interest

The authors declare no conflict of interest.

Supplementary information

Supplementary Information accompanies the paper on the EJCN website (http://www.nature.com/ejcn)

References

1. Libby P. Inflammation in Atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012;32(9):2045-51.

2. Libby P, Ridker PM. Inflammation and atherosclerosis: role of C-reactive protein in risk assessment. *Am J Med* 2004;116 Suppl 6A:9s-16s.

3. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature reviews Immunology* 2006;6(10):772-83.

4. Visser M, Bouter LM, McQuillan GM, Wener MH, Harris TB. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 1999;282(22):2131-5.

5. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. *J Clin Invest* 2003;111(12):1805-12.

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(8):1323-8.

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(11):2541-6.

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(6):791-5.

10. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? *Br J Clin Pharmacol* 2013;75(3):645-62.

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(6):2070S-84S.

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(5):367-72.

13. Nakamura MT, Nara TY. Structure, function, and dietary regulation of Δ6, Δ5, and Δ9 desaturases. *Annu Rev Nutr* 2004;24:345-76.

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(2):439-46.

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

526 inflammation: the Multi-Ethnic Study of Atherosclerosis. *Int J Obes*
527 *(Lond)* 2012;36(6):797-804.
528 16. Kaikkonen JE, Kresanov P, Ahotupa M, Jula A, Mikkila V,
529 Viikari JS, et al. High serum n6 fatty acid proportion is associated
530 with lowered LDL oxidation and inflammation: the Cardiovascular
531 Risk in Young Finns Study. *Free Radic Res* 2014;48(4):420-6.
532 17. Muka T, Kiefte-de Jong JC, Hofman A, Dehghan A,
533 Rivadeneira F, Franco OH. Polyunsaturated fatty acids and serum C-
534 reactive protein: the Rotterdam study. *Am J Epidemiol*
535 2015;181(11):846-56.
536 18. Klein-Platat C, Drai J, Oujaa M, Schlienger JL, Simon C.
537 Plasma fatty acid composition is associated with the metabolic
538 syndrome and low-grade inflammation in overweight adolescents.
539 *Am J Clin Nutr* 2005;82(6):1178-84.
540 19. González-Gil EM, Santabárbara J, Siani A, Ahrens W, Sioen
541 I, Eiben G, et al. Whole-blood fatty acids and inflammation in
542 European children: the IDEFICS Study. *Eur J Clin Nutr*
543 2016;70(7):819-23.
544 20. Heinrich J, Bolte G, Holscher B, Douwes J, Lehmann I,
545 Fahlbusch B, et al. Allergens and endotoxin on mothers' mattresses
546 and total immunoglobulin E in cord blood of neonates. *Eur Respir J*
547 2002;20(3):617-23.
548 21. Glaser C, Rzehak P, Demmelmair H, Klopp N, Heinrich J,
549 Koletzko B, et al. Influence of FADS Polymorphisms on Tracking of
550 Serum Glycerophospholipid Fatty Acid Concentrations and
551 Percentage Composition in Children. *PLoS ONE* 2011;6(7):e21933.

552 22. Glaser C, Demmelmair H, Sausenthaler S, Herbarth O,
553 Heinrich J, Koletzko B. Fatty acid composition of serum
554 glycerophospholipids in children. *J Pediatr* 2010;157(5):826-31.e1.
555 23. Standl M, Thiering E, Demmelmair H, Koletzko B, Heinrich
556 J. Age-dependent effects of cord blood long-chain PUFA
557 composition on BMI during the first 10 years of life. *Br J Nutr*
558 2014;111(11):2024-31.
559 24. Glaser C, Demmelmair H, Koletzko B. High-throughput
560 analysis of fatty acid composition of plasma glycerophospholipids. *J*
561 *Lipid Res* 2010;51(1):216-21.
562 25. Lands B. Consequences of essential fatty acids. *Nutrients*
563 2012;4(9):1338-57.
564 26. Simopoulos AP. The importance of the omega-6/omega-3
565 fatty acid ratio in cardiovascular disease and other chronic diseases.
566 *Experimental biology and medicine (Maywood)*. 2008;233(6):674-88.
567 27. Marventano S, Kolacz P, Castellano S, Galvano F, Buscemi
568 S, Mistretta A, et al. A review of recent evidence in human studies of
569 n-3 and n-6 PUFA intake on cardiovascular disease, cancer, and
570 depressive disorders: does the ratio really matter? *Int J Food Sci Nutr*
571 2015;66(6):611-22.
572 28. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel
573 LL, Appel LJ, et al. Omega-6 Fatty Acids and Risk for
574 Cardiovascular Disease. A Science Advisory From the American
575 Heart Association Nutrition Subcommittee of the Council on
576 Nutrition, Physical Activity, and Metabolism; Council on
577 Cardiovascular Nursing; and Council on Epidemiology and
578 Prevention. *Circulation* 2009;119(6):902-7.

29. Ohnishi H, Saito Y. Eicosapentaenoic acid (EPA) reduces cardiovascular events: relationship with the EPA/arachidonic acid ratio. *J Atheroscler Thromb* 2013;20(12):861-77.

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 Research International* 2015;2015:11.

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(4):941-9.

32. Vessby B, Gustafsson IB, Tengblad S, Boberg M, Andersson A. Desaturation and elongation of Fatty acids and insulin action. *Ann N Y Acad Sci* 2002;967:183-95.

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. Fourth ed: Springer, New York; 2002.

35. Shine B, de Beer FC, Pepys MB. Solid phase radioimmunoassays for human C-reactive protein. *Clin Chim Acta* 1981;117(1):13-23.

36. Kleiner G, Marcuzzi A, Zanin V, Monasta L, Zauli G. Cytokine Levels in the Serum of Healthy Subjects. *Mediators of Inflammation* 2013;2013:6.

606 37. Vittinghoff E, McCulloch CE. Relaxing the Rule of Ten
607 Events per Variable in Logistic and Cox Regression. *Am J Epidemiol*
608 2007;165(6):710-8.
609 38. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR.
610 A simulation study of the number of events per variable in logistic
611 regression analysis. *J Clin Epidemiol* 1996;49(12):1373-9.
612 39. Black S, Kushner I, Samols D. C-reactive Protein. *J Biol*
613 *Chem* 2004;279(47):48487-90.
614 40. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger
615 T, Trullenque R, et al. Interleukin-6 is the major regulator of acute
616 phase protein synthesis in adult human hepatocytes. *FEBS Lett*
617 1989;242(2):237-9.
618 41. Bode JG, Albrecht U, Häussinger D, Heinrich PC, Schaper F.
619 Hepatic acute phase proteins–regulation by IL-6-and IL-1-type
620 cytokines involving STAT3 and its crosstalk with NF-κB-dependent
621 signaling. *Eur J Cell Biol* 2012;91(6):496-505.
622 42. Ganter U, Arcone R, Toniatti C, Morrone G, Ciliberto G.
623 Dual control of C-reactive protein gene expression by interleukin-1
624 and interleukin-6. *EMBO J* 1989;8(12):3773-9.
625 43. Ford ES. C-reactive protein concentration and cardiovascular
626 disease risk factors in children: findings from the National Health
627 and Nutrition Examination Survey 1999-2000. *Circulation*
628 2003;108(9):1053-8.
629 44. Decsi T, Kennedy K. Sex-specific differences in essential
630 fatty acid metabolism. *Am J Clin Nutr* 2011;94(6 Suppl):1914S-9S.

631 45. Burdge GC, Wootton SA. Conversion of alpha-linolenic acid
632 to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in
633 young women. *Br J Nutr* 2002;88(4):411-20.
634 46. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated
635 fatty acids and inflammatory mediator production. *Am J Clin Nutr*
636 2000;71(1 Suppl):343s-8s.
637 47. King DE, Egan BM, Geesey ME. Relation of dietary fat and
638 fiber to elevation of C-reactive protein. *Am J Cardiol*
639 2003;92(11):1335-9.
640 48. Weigert C, Brodbeck K, Staiger H, Kausch C, Machicao F,
641 Haring HU, et al. Palmitate, but not unsaturated fatty acids, induces
642 the expression of interleukin-6 in human myotubes through
643 proteasome-dependent activation of nuclear factor-kappaB. *J Biol*
644 *Chem* 2004;279(23):23942-52.
645 49. Ajuwon KM, Spurlock ME. Palmitate Activates the NF-κB
646 Transcription Factor and Induces IL-6 and TNFα Expression in 3T3-
647 L1 Adipocytes. *J Nutr* 2005;135(8):1841-6.
648 50. Enzenbach C, Kroger J, Zietemann V, Jansen EH, Fritsche
649 A, Doring F, et al. Erythrocyte membrane phospholipid
650 polyunsaturated fatty acids are related to plasma C-reactive protein
651 and adiponectin in middle-aged German women and men. *European*
652 *journal of nutrition* 2011;50(8):625-36.
653 51. De Caterina R, Liao JK, Libby P. Fatty acid modulation of
654 endothelial activation. *Am J Clin Nutr* 2000;71(1 Suppl):213s-23s.
655 52. Lands B. Historical perspectives on the impact of n-3 and n-6
656 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. *Journal of the Academy of Nutrition and Dietetics* 2012;112(7):1029-41, 41.e1-15.

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;4:151-9.

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. *Nutrition & Metabolism* 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(5):1003-12.

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(4):945-52.

58. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Current opinion in clinical nutrition and metabolic care* 2002;5(2):127-32.

59. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO food and nutrition paper. 2010;91:1-166.

60. Riboli E, Ronnholm H, Saracci R. Biological markers of diet. *Cancer Surv* 1987;6(4):685-718.

683 61. Rise P, Eligini S, Ghezzi S, Colli S, Galli C. Fatty acid
684 composition of plasma, blood cells and whole blood: relevance for
685 the assessment of the fatty acid status in humans. *Prostaglandins*
686 *Leukot Essent Fatty Acids* 2007;76(6):363-9.
687 62. Sergeant S, Ruczinski I, Ivester P, Lee TC, Morgan TM,
688 Nicklas BJ, et al. Impact of methods used to express levels of
689 circulating fatty acids on the degree and direction of associations
690 with blood lipids in humans. *Br J Nutr* 2016;115(2):251-61.
691 63. Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ,
692 Kompauer I, et al. Common genetic variants of the FADS1 FADS2
693 gene cluster and their reconstructed haplotypes are associated with
694 the fatty acid composition in phospholipids. *Hum Mol Genet*
695 2006;15(11):1745-56.
696 64. Steffen LM, Vessby B, Jacobs DR, Jr., Steinberger J, Moran
697 A, Hong CP, et al. Serum phospholipid and cholesteryl ester fatty
698 acids and estimated desaturase activities are related to overweight
699 and cardiovascular risk factors in adolescents. *International Journal*
700 *of Obesity* 2008;32(8):1297-304.

701

Figure Legends

Fig. 1 Study participants

[a]hs-CRP > 1mg/dl or IL-6 > 20pg/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: e.g. diabetes, anorexia, coeliac disease, cancer.

Fig. 2 Odds ratio (OR) and 95% confidence interval (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 *.

Table1. Descriptive characteristics of study participants

	All N=958	**Males** N=520	**Females** N=438	**P-value[g]**
	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[a]				
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)[b]	927 (96.8)	502 (96.5)	425 (97.0)	0.805
Screen-timc[c]				
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)[d]	429 (44.8)	139 (26.7)	290 (66.2)	**<0.001**
hs-CRP groups[e]				
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[f]				
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 (sd) or median (25th;75th perc.)	**mean (sd) or median (25th;75th perc.)**	**mean (sd) 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
n6/n3	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**

Values are presented as counts (%) for categorical variables, mean (standard deviation) for normally distributed continuous variables, and median (25th;75th percentile) for non-normally distributed continuous variables. [a]Highest level achieved (low: <10th grade; medium: 10th grade; high: >10th grade). [b]Whether the child was ever breastfed (yes: ≥1 month). [c]Self-reported h/day spent on screen activities (Low: ≤1h in winter and ≤2h in summer; Medium: >1h in winter or >2h in summer; High: >1h in winter and >2h in summer). [d]Females: estradiol >18.4pmol/L; Males: testosterone >0.09nmol/L. [e](I) hs-CRP < 0.02mg/dl; (II) hs-CRP ≥ 0.02mg/dl and < 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (< 0.11mg/dl in girls; < 0.09mg/dl in boys); and (III) hs-CRP ≥ 75th sex-specific percentile of those with hs-CRP ≥ 0.02 mg/dl (≥ 0.11mg/dl in girls; ≥ 0.09mg/dl in boys). [f](I) IL-6 ≤ 1.5pg/ml; (II) IL-6 > 1.5pg/ml and < 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (< 4.26pg/ml in girls; < 3.93pg/ml in boys); and (III) IL-6 ≥ 75th sex-specific percentile of those with IL-6 > 1.5pg/ml (≥ 4.26pg/ml in girls; ≥ 3.93pg/ml in boys). [g]Comparison between males and females: tested by Student's t-test (means) or by Wilcoxon rank sum test (medians) for continues variables, and by Pearson's Chi-squared Test for categorical variables. Significant p-values are marked in bold (p<0.05)

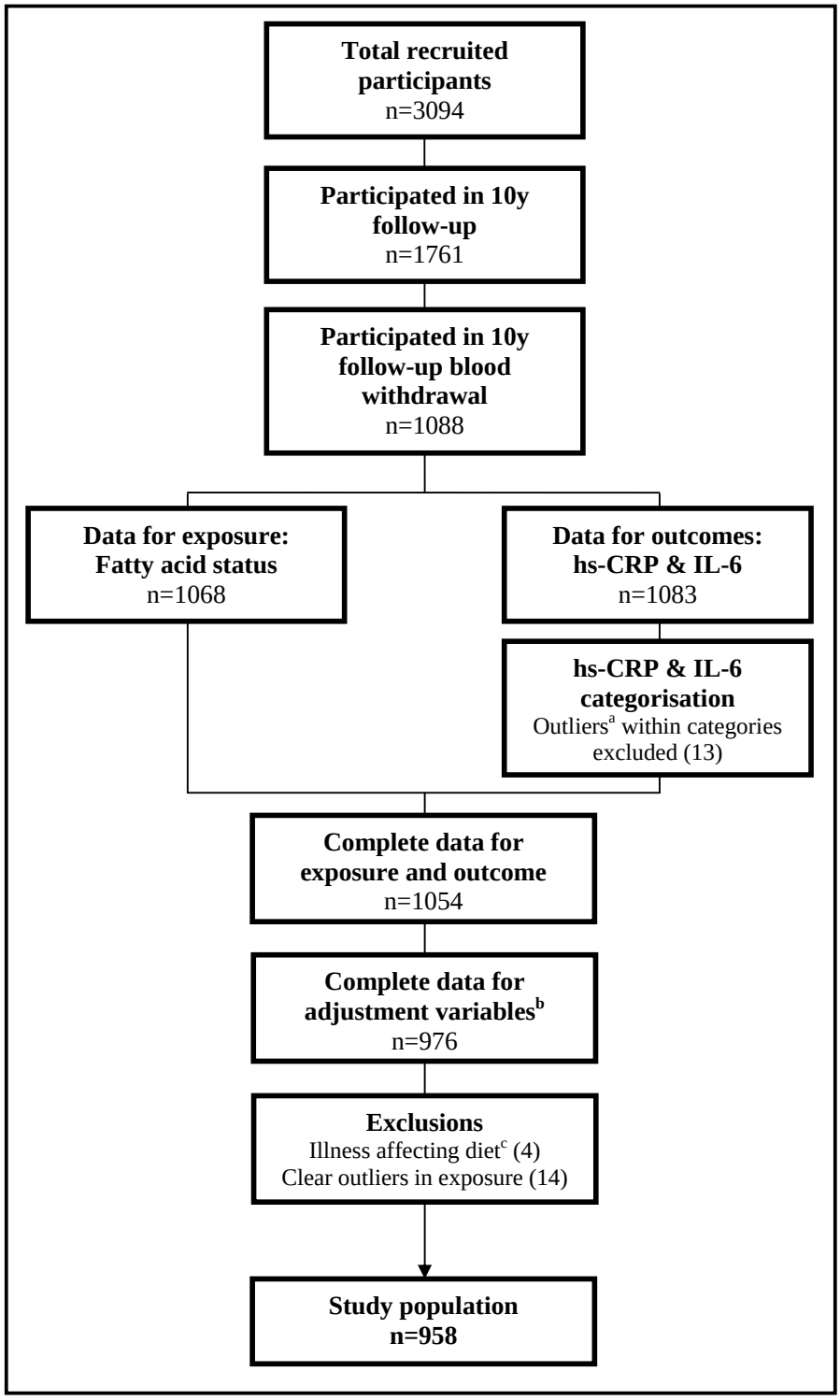
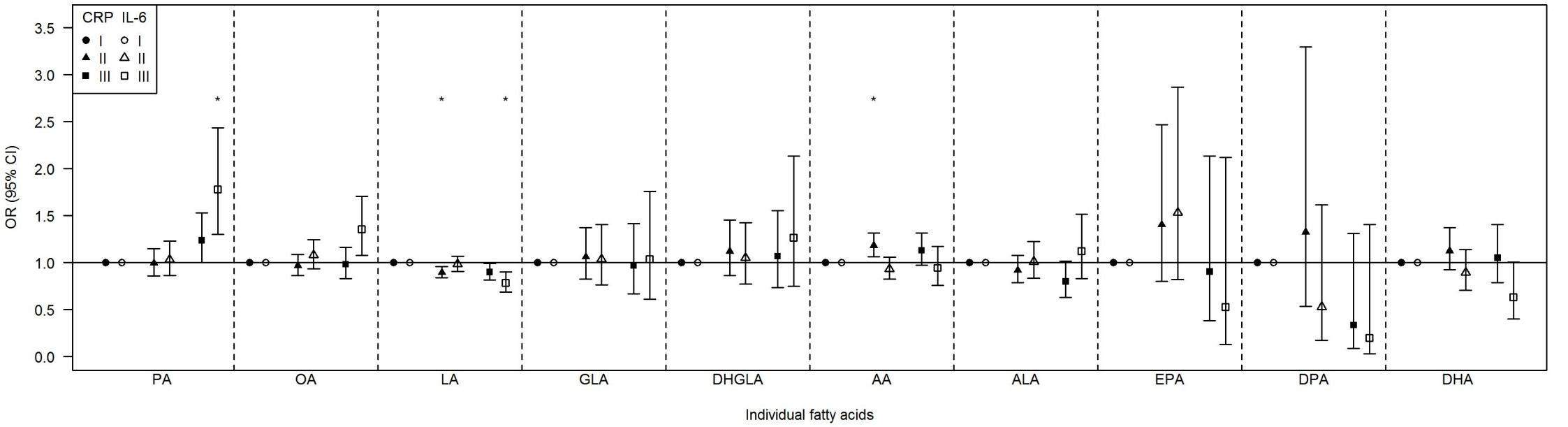

Total recruited participants
n=3094
Participated in 10y follow-up
n=1761
Participated in 10y follow-up blood withdrawal
n=1088
Data for exposure: Fatty acid status
n=1068
Data for outcomes: hs-CRP & IL-6
n=1083
hs-CRP & IL-6 categorisation
Outliers[a] within categories excluded (13)
Complete data for exposure and outcome
n=1054
Complete data for adjustment variables[b]
n=976
Exclusions
Illness affecting diet[c] (4)
Clear outliers in exposure (14)
Study population
n=958

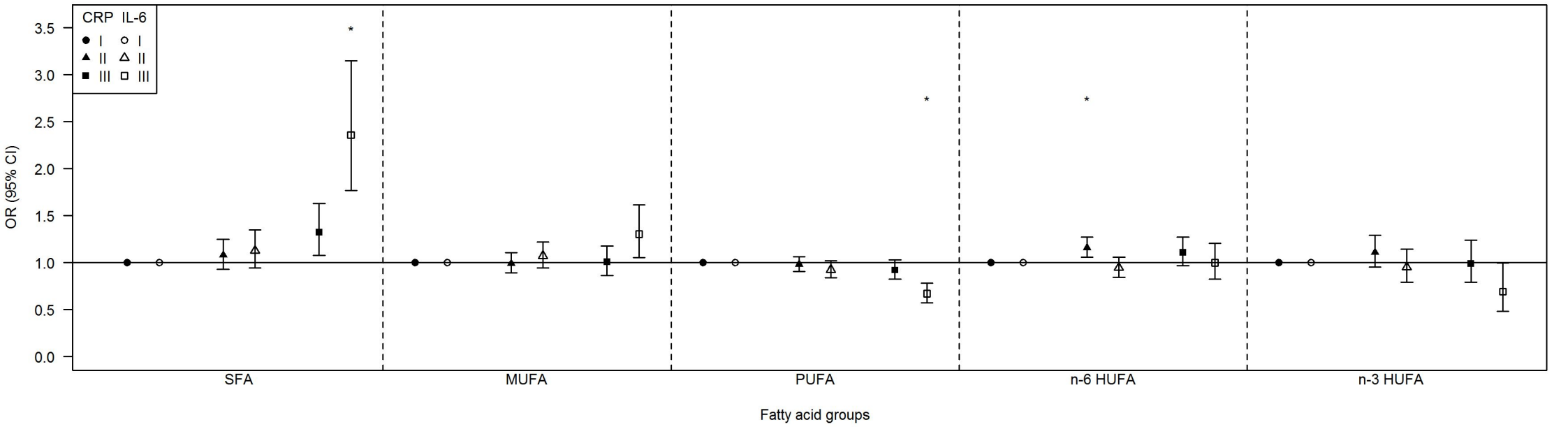
Figure 1

Table 2. Odds ratio and 95% confidence interval assessing the association of fatty acid exposures with hs-CRP and IL-6 categories. 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.

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
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**

[a]OR for these variables refer to changes of 0.1 units. Significant associations are marked in bold (Bonferroni corrected p-value <0.0025)


CRP IL-6
I I
II II
III III
OR (95% CI)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PA
OA
LA
GLA
DHGLA
AA
ALA
EPA
DPA
DHA
Individual fatty acids


CRP IL-6
I I
II II
III III
OR (95% CI)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SFA
MUFA
PUFA
n-6 HUFA
n-3 HUFA
Fatty acid groups

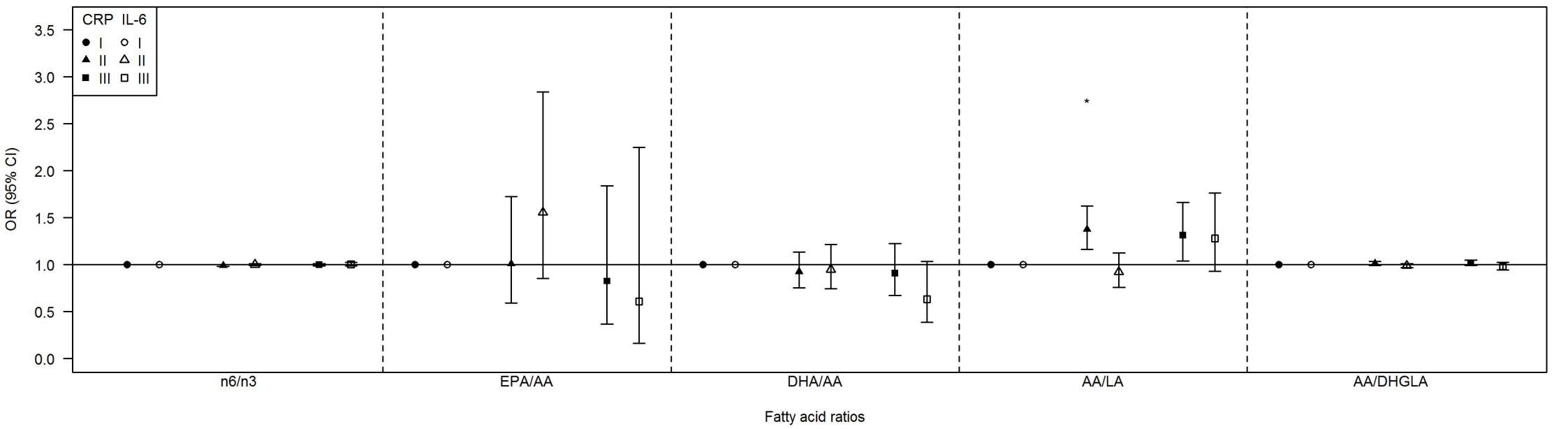

CRP IL-6
I I
II II
III III
OR (95% CI)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
n6/n3
EPA/AA
DHA/AA
AA/LA
AA/DHGLA
Fatty acid ratios

Figure 2