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ORIGINAL ARTICLE

Whole-blood fatty acids and inflammation in European children: the IDEFICS Study

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BACKGROUND/OBJECTIVES: Fatty acids are hypothesized to influence cardiovascular disease risk because of their effect on inflammation. The aim of this study is to assess the relationship between whole-blood fatty acids (WBFAs) and high-sensitivity C-reactive protein (hs-CRP) in European children.

SUBJECTS/METHODS: A total of 1401 subjects (697 boys and 704 girls) aged between 2 and 9 years from the IDEFICS (*I*dentification and prevention of *D*ietary- and lifestyle-induced health *EF*fects in *C*hildren and infant*S*) study were measured in this cross-sectional analysis. The sample was divided into three categories of hs-CRP. Associations between WBFA and hs-CRP were assessed by logistic regression models adjusting for body mass index (BMI), country, age, breastfeeding, mother's education and hours of physical activity.

RESULTS: Linoleic acid (LA) ($P = 0.013$, 95% confidence interval (CI): 0.822–0.977) and sum of n-6 WBFA ($P = 0.029$, 95% CI: 0.866–0.992) concentrations were associated with lower concentrations of hs-CRP in boys. In girls, a high ratio of eicosapentaenoic acid (EPA)/arachidonic acid (AA) was associated ($P = 0.018$, 95% CI: 0.892–0.989) with lower hs-CRP concentrations. In contrast, sum of blood n-6 highly unsaturated fatty acids ($P = 0.012$, 95% CI: 1.031–1.284), AA ($P = 0.007$, 95% CI: 1.053–1.395) and AA/LA ratio ($P = 0.005$, 95% CI: 1.102–1.703) were associated ($P < 0.05$) with higher concentrations of hs-CRP in girls.

CONCLUSIONS: The n-6 WBFAs (sum of n-6 FA and LA) were associated with lower hs-CRP in boys and with higher hs-CRP in girls (AA, sum of n-6 highly unsaturated and AA/LA ratio). More studies are needed to identify the optimal levels of WBFAs to avoid low-grade inflammation in children considering the differences by sex and BMI.

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INTRODUCTION

Low-grade chronic inflammation is related with obesity[1–3] and with the onset and development of atherosclerosis.[1,4] Atherosclerosis development is characterized by an interaction between vascular endothelial cells and circulating leukocytes,[5] especially in early stages of the process. High-sensitivity C-reactive protein (hs-CRP) is the most widely used biomarker of inflammation associated with adiposity[6–9] and atherosclerosis progression, as assessed by intima–media thickness, even in children.[7] In previous literature, food, nutrient intake and dietary patterns have also been shown to be associated with hs-CRP, as a marker of inflammation, and cardiovascular disease (CVD) risk factors.[10–12]

Among dietary factors associated with inflammation, fatty acids (FAs) seem to play a relevant role.[12] Inflammatory response can be modulated by some FAs by different mechanisms, such as transcriptional downregulation of proinflammatory cytokines and vascular surface expression of endothelial leukocyte adhesion molecules.[13] However, not all FAs play the same role in the inflammatory process. Consumption of polyunsaturated fatty acids (PUFAs), especially dietary n-3 PUFAs, has been suggested to reduce inflammation.[14–16] N-3 FA blood levels, especially long-chain FAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are related with lower CVD risk.[17] Long-chain n-3 PUFAs decrease the production of inflammatory mediators, eicosanoids, cytokines and reactive oxygen species, and also the expression of adhesion molecules and are precursors of resolvins that are considered anti-inflammatory mediators.[18] In addition, plasma n-3 FAs have been independently associated with lower CRP concentrations.[19] In contrast, the most relevant long-chain n-6 PUFA, the arachidonic acid (AA), has been hypothesized to have proinflammatory properties by generating lipid mediators that cause vessel inflammation and endothelial and platelet dysfunction.[20]

An important issue in studies aimed to investigate associations between FA and inflammation/CVD is the assessment of the FA status on an individual basis. There is considerable individual variability in FA concentrations depending on dietary intake, absorption and metabolism, mainly because of genetic variations.[21] Therefore, the assessments of FA in blood (serum, plasma, erythrocytes, whole blood)

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provide an accurate measure of the FA status in contrast with the estimation of dietary intake.[17] Whole-blood measurement of FA is a recently developed method and is adequate for epidemiological studies as it is valid, noninvasive and time and cost saving.[22] This method, which reflects dietary intake only using fingertip puncture, presented no significant differences with samples obtained from venous blood.[22]

The aim of this exploratory study is to assess the relationship between whole-blood FA (WBFA) levels and hs-CRP to identify WBFAs associated with inflammation in European children.

MATERIALS AND METHODS

Subjects

IDEFICS (*I*dentification and prevention of *D*ietary- and lifestyle-induced health *EF*fects in *C*hildren and infant*S*) is a large European multicenter study on childhood obesity. A total of 16 224 children aged 2–9 years were recruited into a population-based baseline survey from 8 European countries (Sweden, Germany, Hungary, Italy, Cyprus, Spain, Belgium and Estonia). Parents reported sociodemographic, behavioral, medical, nutritional and other lifestyle information for their children and families. Examinations of children included anthropometry, blood pressure, physical fitness, physical activity, DNA from saliva and biochemical markers. Detailed information regarding design, characteristics and participation can be found elsewhere.[23]

This study was conducted according to the Declaration of Helsinki. Approvals for the ethics committee were obtained from the local authorities in each center. All children were informed and provided oral consent while the parents gave their written consent.

Out of the total of 16 228 children, 9601 provided blood samples. The hs-CRP was measured in 9038 of these children but 2647 were excluded as they had taken medication the week before blood drawing. On the other hand, 2600 of the total 16 228 children had the WBFA measured. Of these, 1413 children met the criteria of having hs-CRP and WBFA measured. Cyprus and Belgium were excluded from the study, as the number of subjects in these countries was very low, 3 and 9 respectively. For the current analysis, 1401 subjects were used (697 boys and 704 girls).

Biochemical analysis

Children were asked to participate, on a voluntary basis, in fasting blood draw. A detailed description of sample collection and analytical procedures can be found elsewhere.[24]

The hs-CRP concentrations were measured in a central laboratory with a high-sensitivity assay using latex-enhanced nephelometry (BN2-Nephelometer, Siemens, Deerfield, IL, USA) and the lower limit of detection of the assay was 0.02 mg/dl.

Whole-blood fatty acids

In the IDEFICS study, 22 WBFAs were measured. The selection of these WBFAs was based on their importance in different aspects: precursors of active compounds, as major components of the diet or for their structural function (cell membrane). For this study, 9 of these single WBFAs were selected because of their relation with the pathways of the inflammatory metabolism: palmitic acid, oleic acid, linoleic acid (LA), γ-linoleic acid, AA, α-linolenic acid, EPA, docosapentaenoic acid and DHA.[5,20,25–28] In addition, n-3 highly unsaturated fatty acids (n-3 HUFA), highly unsaturated n-6 (n-6 HUFA) and the ratio between n-3 HUFA and n-3 HUFA+n-6 HUFA (×100 n-3 HUFA) were measured. The ratios calculated are, in some cases, indexes of WBFA conversion (from the precursor to the product, for example, LA/AA) or a simple ratio (n-3/n-6) with possible implications in the synthesis of eicosanoids.

WBFA analysis

The drop of blood was obtained by punching the fingertip with a lancet from an automatic lancing device, and blood was absorbed on a strip of paper for chromatography (Schleicher Schuell, Dassel, Germany; Chromatography Paper, preparative, 165 gsm). If the children agreed to venous puncture, a drop of blood was taken from the sample to be applied to the WBFA strip. The amount of blood collected fluctuated between 15 and 75 μg (equivalent to the same values in μl). The strips with the drop of blood were either immediately processed or stored at 4 °C in individual cellophane envelopes with airtight closure for better storage. Further information can be found elsewhere.[29]

Covariates

The models were adjusted for potential confounders, selected on the basis of previously published associations with hs-CRP or associations with exposures or outcomes in the present analysis. Covariates used for the analysis were: age, body mass index (BMI), country, education of the mother, breastfeeding (BF) and self-reported hours of physical activity (PA) in a sport club per week. The International Standard Classification of Education (ISCED) was used to report mother's education.[30] BF was treated as a dichotomous variable, being yes if the mother stated months of BF, or no if the child was not breastfed. In order to maximize the sample, self-reported hours of PA in a sport club were taken. In the IDEFICS study, children's weekly hours in sports club activities was significantly correlated with data from accelerometers.[31] BMI was calculated using measured weight and height.[32]

Statistical analysis

Kolmogorov–Smirnov test was used to contrast normality. The analyses were performed separately in boys and girls, as sex was an effect modifier when assessing the associations.

Because ~50% of children had values less than the minimal detectable concentration, in this analysis hs-CRP is treated as a categorical rather than a continuous variable. The following three hs-CRP cutoffs were defined to categorize hs-CRP levels in our population: (1) hs-CRP under the detecti on limit (<0.02 mg/dl); (2) hs-CRP >0.02 mg/dl and $<$75th sex-specific percentile of those with hs-CRP values over the detection limit (<0.21 mg/dl in boys and 0.22 mg/dl in girls); and (3) hs-CRP $>$75th sex-specific percentile of those with hs-CRP values over the detection limit (>0.21 mg/dl in boys and >0.22 mg/dl in girls).[33] Analysis of covariance was performed, separately in boys and girls, to assess the differences in mean WBFA concentrations between categories of hs-CRP. The covariables entered in the model were the continuous ones: age, BMI and self-reported hours of PA per week. The *post hoc* comparisons between hs-CRP groups were conducted with a Bonferroni correction applied.

To assess the association between hs-CRP groups and concentration of each WBFA, we used ordinal logistic regression models. In order to explore mechanisms of the association, we used two hierarchical models in which we controlled for potential confounders (age, mother education, country, BMI, BF and self-reported hours of PA in sports club per week). Model 1, presented as Supplementary Material, included each WBFA, age, mother education and country. Model 2 was a fully adjusted model including BMI, BF and self-reported hours of PA in sports club per week in addition to the covariates assessed in model 1.

The odds ratio (OR) of the WBFA presented the percentage of being in the upper CRP group by increasing the WBFA concentration in 1 unit. In contrast, the ORs of the ratios, ratio DHA/AA, ratio EPA/AA, ratio AA/LA, ratio AA/dihommo-γ-linoleic acid and ratio n-6/n-3, showed the percentage of being in the upper CRP group by increasing the ratio in 0.1 units.

Data were managed and analyzed with the IBM SPSS Statistics v.19 (IBM Corp., New York, NY, USA, 2010).

RESULTS

Sample characteristics

Baseline characteristics by sex and distribution by country of participants are shown in Table 1. The mean serum concentration of hs-CRP was significantly higher in girls than in boys ($P<0.001$); in contrast, boys had significantly higher concentrations of some WBFAs such as docosapentaenoic acid ($P<0.001$), the sum of the n-6 highly unsaturated WBFA ($P<0.001$) and the ratio AA/LA ($P<0.001$).

In addition, analysis of covariance was performed using continuous covariables, BMI, age and self reported hours of PA in a sports club, to assess the differences between mean concentrations of each WBFA and the three categories of hs-CRP by gender. This analysis is presented in Supplementary Tables S1 and S2.

Table 1. Descriptive characteristics of the study participants

	Boys, n = *697*		*Girls,* n = *704*		P-*value*
Country	*n* (%)		*n* (%)		
Estonia	38 (5.5%)		47 (6.7%)		—
Germany	190 (27.3%)		201 (28.6%)		—
Hungary	76 (10.9%)		89 (12.6%)		—
Italy	265 (38%)		260 (36.9%)		—
Spain	78 (11.2%)		55 (7.8%)		—
Sweden	50 (7.2%)		52 (7.4%)		—
Mother's educational level	*n* (%)		*n* (%)		
ISCED level 1	46 (7.1%)		52(8%)		
ISCED level 2	173 (26.8%)		153 (23.5%)		
ISCED level 3	245 (37.9%)		255 (39.2%)		
ISCED level 4	61 (9.4%)		661(10.2%)		
ISCED level 5	121 (18.7%)		124 (19.1%)		
Breast feeding (yes), *n* (%)	376 (53.9%)		383 (54.4%)		
Hs-CRP groups					
CRP I	260 (37.3%)		179 (25.4%)		
CRP II	329 (47.2%)		397 (56.5%)		
CRP III	108 (15.5%)		128 (18.2%)		
	Mean	s.d.	Mean	s.d.	
Age (years)	6.41	1.72	6.46	1.71	0.442
Physical activity[a] (h)	1.13	1.58	1.10	1.53	0.703
BMI (kg/m^2)	18.22	3.56	18.09	3.34	0.780
hs-CRP (mg/dl)	0.14	0.32	0.16	0.36	< **0.001**
Palmitic acid (% of total FA)	25.72	1.42	25.76	1.42	0.610
Oleic acid (% of total FA)	18.47	2.04	18.71	2.15	**0.035**
Linoleic acid (% of total FA)	18.1	2.0	18.3	2.04	0.065
GLA (% of total FA)	0.23	0.08	0.22	0.08	**0.006**
Arachidonic acid (% of total FA)	7.61	1.34	7.41	1.38	**0.006**
ALA (% of total FA)	0.19	0.07	0.2	0.08	0.976
EPA (% of total FA)	0.26	0.09	0.26	0.09	0.173
DPA (% of total FA)	0.54	0.16	0.51	0.15	< **0.001**
DHA (% of total FA)	1.19	0.41	1.15	0.41	**0.030**
Sum n-3 HUFA (% of total FA)	2.01	0.59	1.92	0.59	**0.007**
Sum n-6 HUFA (% of total FA)	10.27	1.79	9.91	1.85	< **0.001**
×100 n-3 HUFA (% of total FA)	16.31	3.57	16.18	3.68	0.390
Ratio DHA/AA	0.15	0.04	0.15	0.04	0.286
Ratio EPA/AA	0.03	0.01	0.03	0.01	0.903
Ratio AA/LA	0.42	0.08	0.4	0.01	< **0.001**
Ratio AA/DHGLA	6.1	1.25	6.3	1.31	**0.004**
Ratio n-6/n-3	13.81	3.97	14.23	4.16	0.055
Sum n-6 (% of total FA)	28.63	2.63	28.5	2.67	0.378
Sum n-3 (% of total FA)	2.21	0.61	2.13	0.61	**0.007**
SFA (% of total FA)	44.36	1.86	44.28	1.86	0.437
MUFA (% of total FA)	24.65	2.37	24.94	2.48	**0.026**
PUFA (% of total FA)	30.83	2.78	30.59	2.87	0.129

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; DHA, docosahexaenoic acid; DHGLA, dihommo-γ-linoleic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GLA, γ-linolenic acid; hs-CRP, high-sensitivity C-reactive protein; ISCED, International Standard Classification of Education; LA, linoleic acid; MUFA, sum of monounsaturated fatty acids; n-3 HUFA, highly unsaturated n-3; n-6 HUFA, highly unsaturated n-6; ×100 n-3 HUFA, ratio between n-3 HUFA and n-3 HUFA+n-6 HUFA; (), PUFA, sum of all polyunsaturated fatty acids; SFA, sum of saturated fatty acids. Shown are percentages, mean values and s.d. values. [a]Self-reported hours of physical activity in a sport club per week. Bold entries indicate significant $P < 0.005$.

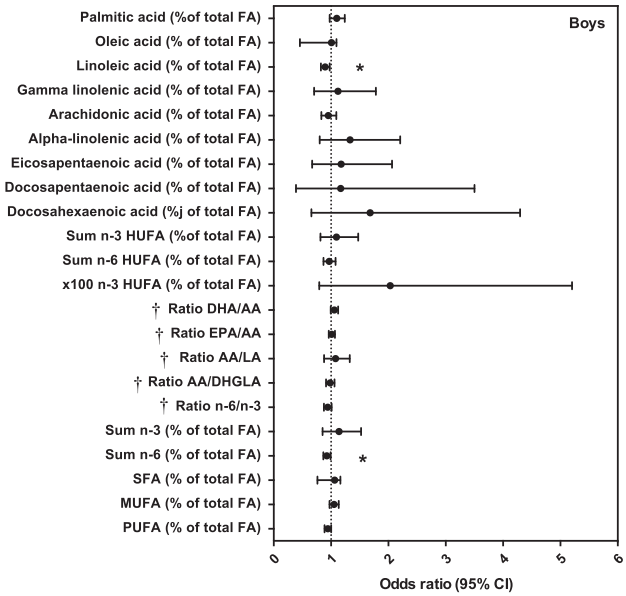

Figure 1. OR (95% CI) assessing the association between hs-CRP categories and blood fatty acid concentrations in boys. Logistic regression model adjusted by: age, education of the mother, country, BMI, BF and self-reported hours of PA in a sports club. DHA, n-3 HUFA, n-6 HUFA, ratio between n-3 HUFA and n-3 HUFA+ n-6 HUFA (×100 n-3 HUFA), AA, LA, dihommo-γ- linoleic acid (DHGLA), sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA) and sum of all polyunsaturated fatty acids.[42] †OR of the ratios, ratio DHA/AA, ratio EPA/AA, ratio AA/LA, ratio AA/DHGLA and ratio n-6/n-3, showed the percentage of being in the upper CRP group by increasing the ratio in 0.1 units. *$P < 0.05$.

Multivariate analysis

In the multivariate analysis, model 1 (Supplementary Tables S3 and S4) and model 2 (Figures 1 and 2), hs-CRP group is considered as dependent variable and each WBFA as independent variable.

In boys, blood concentrations of some WBFAs were significantly associated with higher concentration of hs-CRP. The highest OR observed in the model 1 (Supplementary Table S3) was the concentration of the n-6 γ-linolenic acid (OR = 1.82, 95% confidence interval (CI): 1.177–2.824, $P = 0.007$), meaning that for each unit increased of this acid, the probability of reaching upper level of hs-CRP group increased by 82% after controlling for age, mother education and country. When BMI, self-reported hours of PA per week in a sport club and BF (Figure 1) were added to model 1, some blood WBFAs decreased the probability of reaching the upper level of hs-CRP, such as in the case of LA (OR = 0.89, 95% CI: 0.822–0.977, $P = 0.013$) and sum of n-6 (OR = 0.92, 95% CI: 0.866–0.992, $P = 0.029$).

In girls (Supplementary Table S3), blood concentration of some WBFAs also significantly increased the probability of reaching the upper level of hs-CRP when increasing the concentration of the WBFA in 1 unit: γ-linolenic acid by 92% (OR = 1.92, 95% CI: 1.240–2.989, $P = 0.004$), AA by 24% (OR = 1.24, 95% CI: 1.089–1.415, $P < 0.001$) and for sum of n-6 HUFAs by 20% (OR = 1.20, 95% CI: 1.084–1.331, $P < 0.001$). In addition, when increasing the ratio AA/LA in 0.1 units, the probability of reaching the upper level of hs-CRP increased by 40% (OR = 1.404, 95% CI: 1.149–1.553, $P = 0.001$). In contrast, increases of 0.1 units in blood concentration of DHA/AA ratio decreased the probability of having a higher concentration of hs-CRP by 6% (OR = 0.939, 95% CI: 0.884–0.998, $P = 0.044$). Some of these relationships were also observed when adding the following covariables: BMI, self-reported hours of PA and BF (Figure 2) to model 1. This was the case for AA (OR = 1.21, 95% CI: 1.053–1.395, $P = 0.007$) the sum of n-6 HUFAs (OR = 1.15, 95% CI: 1.031–1.284, $P = 0.012$) and AA/ LA ratio (OR = 1.37, 95% CI: 1.102–1.703, $P = 0.005$) by increasing the concentration in 0.1 units. In contrast, blood EPA/AA ratio (OR = 0.939, 95% CI: 0.892–0.989, $P = 0.018$) significantly decreased the probability of having a higher concentration of hs-CRP.

Out of all covariables, in the ordinal logistic regression, BMI, as continuous variable, showed association with some of the WBFAs, specifically n-3 HUFAs ($P < 0.001$) and sum of n-3 ($P < 0.001$).

DISCUSSION

In a subsample of European children participating in the baseline IDEFICS cross-sectional study, associations were observed, stratifying by sex, between WBFA and hs-CRP concentrations after controlling by a set of potential confounders.

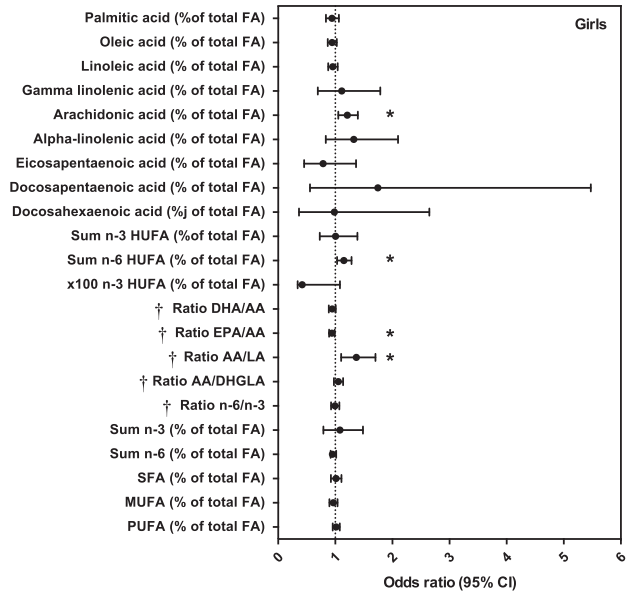

Figure 2. OR (95% CI) assessing the association between hs-CRP categories and blood fatty acid concentrations in girls. Logistic regression model adjusted by: age, education of the mother, country, BMI, BF and self-reported hours of PA in a sports club. DHA, n-3 HUFA, n-6 HUFA, ratio between n-3 HUFA and n-3 HUFA+ n-6 HUFA (×100 n-3 HUFA), AA, LA, dihommo-γ- linoleic acid (DHGLA), sum of saturated fatty acids (SFA), sum of monounsaturated fatty acids (MUFA) and sum of all polyunsaturated fatty acids.[42] †OR of the ratios, ratio DHA/AA, ratio EPA/AA, ratio AA/LA, ratio AA/DHGLA and ratio n-6/n-3, showed the percentage of being in the upper CRP group by increasing the ratio in 0.1 units. *$P < 0.05$.

Among the main findings of our study, LA concentration and sum of n-6 WBFA concentrations were inversely associated with hs-CRP concentrations in boys, whereas EPA/AA ratio was inversely associated with hs-CRP in girls. In addition, DHA/AA ratio was inversely associated with hs-CRP when adjusting by age, education and country in the logistic regression for both sexes.

These results found in boys are in line with previous studies: total PUFA concentrations have anti-inflammatory properties, as the presence of double bonds, regardless of the n-3 or n-6 bond position, seems to be crucial to the modulation of endothelial–leukocyte interaction.[5]

In girls, the DHA/AA and EPA/AA ratios were inversely associated with hs-CRP concentrations. In previous studies, the blood n-3 to n-6 PUFA ratio and DHA/AA ratio were negatively associated with change in plaque volume in patients with coronary artery disease,[34] whereas EPA/AA ratio was inversely related with major coronary events in a general Japanese population.[35] EPA has been described as a clinically relevant measurement[17] and, along with DHA, has the opposite influence with AA.[27,36] AA is the main n-6 long-chain FA and is a source of prostaglandins and leukotrienes, mediators that cause vessel inflammation and endothelial and platelets dysfunction, and has been related with ischemic heart disease.[20] EPA and DHA prevent the conversion of AA to pro-inflammatory eicosanoids and the formation of anti-inflammatory compounds.[37] This would shift the production of inflammatory eicosanoids synthesized from n-6 PUFA to n-3 PUFA, less inflammatory than their AA-derived eicosanoid compounds.[36]

Furthermore, in girls, the WBFAs associated with hs-CRP concentrations were: sum of n-6 HUFAs, AA and ratio AA/LA. As mentioned before, AA has a role in inflammation and immune function as it is precursor of prostaglandins, leukotrienes and related compounds.[38] In our study, high AA/LA ratios, index of the conversion rate of LA to AA, was positively correlated with enhanced proinflammatory conditions, in support of the concept that enhanced production of the proinflammatory AA may play a role.

Differences found by gender are in line with previous data suggesting more efficient PUFA metabolism in women than in men. Apparently, estrogens stimulates whereas testosterone inhibits the conversion of short chained FAs to their longer chain derivates, although the effect seems to be moderate.[39] These gender differences are higher regarding the synthesis of long-chain n-3 FA, especially DHA, in adults and adolescents.[40,41]

Among all the covariables, BMI showed the strongest association with hs-CRP concentrations in children, confirming previous literature[8] that suggest that BMI should be taking into account when assessing hs-CRP and WBFA.

The strengths of the study are the use of standardized data from children living in six European countries and the population, as there is no literature assessing the relationship between WBFA and inflammation in healthy young European children. In addition, the use of WBFA concentrations is a strength as, in addition to dietary intake, there are physiological and genetic mechanisms explaining the large variability on body FA status. Additionally, blood FA concentrations are an important clinical measurement to assess CVD risk.[17] The first limitation of the study is the use of hs-CRP alone, as the measurement of additional inflammatory biomarkers would have been useful to explain the mechanisms for these associations. Another limitation is that country is not considered separately for sample size reasons; however, country as covariable is considered in both models of the logistic ordinal regression. Finally, the cross-sectional study design does not allow examining causality.

In conclusion, the results of this exploratory study suggest that n-6 WBFAs presented more associations with hs-CRP in children than n-3 in both sexes. The n-6 WBFAs, specifically sum of n-6 FA and LA, were associated with lower hs-CRP in boys, and AA, sum of n-6 highly unsaturated and AA/LA ratio with higher hs-CRP in girls. In addition, higher EPA/AA and DHA/AA concentrations ratios were associated with low serum hs-CRP concentrations across the different analyses in girls. Furthermore, these results suggest that sex and BMI should be taken into account when assessing blood concentrations of WBFA. More studies in children are needed to identify the optimal levels of WBFA to avoid systemic low-grade inflammation that could lead to cardiovascular diseases in later life.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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