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ORIGINAL CONTRIBUTION

Erythrocyte membrane phospholipid polyunsaturated fatty acids are related to plasma C-reactive protein and adiponectin in middle-aged German women and men

Cornelia Enzenbach · Janine Kröger · Vera Zietemann · Eugène H. J. M. Jansen · Andreas Fritsche · Frank Döring · Heiner Boeing · Matthias B. Schulze

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Abstract
Purpose Modulation of circulating inflammatory markers and adiponectin may link PUFA to risk of diabetes and cardiovascular diseases. We investigated erythrocyte n-6 and n-3 PUFA in relation to plasma C-reactive protein (CRP) and adiponectin, and whether the Pro12Ala polymorphism in the PPARγ2 gene (PPARG2) modified these associations.

Methods We conducted a cross-sectional analysis among 1,222 women and 758 men participating in the EPIC-Potsdam study.

Results Most notably, in both sexes, higher linoleic acid (LA) was related to lower CRP (geometric mean outcome [mg/L], quintile 1, quintile 5, p for trend ≤ 0.01 unless otherwise stated: 0.95, 0.61 [women], 0.67, 0.51 [men]) and higher adiponectin (7.9, 9.1 [women], 5.3, 6.1 [men]), whereas higher γ-linolenic acid (GLA) and dihomo-γ-linolenic acid (DGLA) were related to higher CRP (GLA: 0.63, 0.92 [women], 0.55, 0.70, $p = 0.08$ [men], DGLA: 0.55, 1.07 [women], 0.52, 0.76 [men]) and lower adiponectin (GLA: 8.6, 8.0 [women], 5.8, 5.4, $p = 0.08$ [men], DGLA: 9.2, 7.9 [women], 5.9, 5.4, $p = 0.08$ [men]) adjusting for age and lifestyle. The associations mostly did neither strongly nor significantly vary by PPARG2 genotype. In women, Pro12Ala appeared to interact with arachidonic acid on CRP ($p = 0.04$), as well as with docosatetraenoic acid on CRP ($p = 0.08$) and adiponectin ($p = 0.02$).

Conclusions Our findings suggest that erythrocyte PUFA, particularly LA and n-6 higher unsaturated fatty acids, are related to circulating CRP and adiponectin. They do not indicate that PUFA strongly interact with the PPARG2 Pro12Ala variant on these risk markers.

Keywords Adiponectin · C-reactive protein · Dihomo-gamma-linolenic acid · Fatty acids · Molecular epidemiology

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C. Enzenbach · V. Zietemann · M. B. Schulze
Public Health Nutrition Unit, Technische Universität München, Freising, Germany

J. Kröger · H. Boeing · M. B. Schulze
Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

J. Kröger · M. B. Schulze (✉)
Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Arthur-Scheunert-Allee 114-116, 14558 Nuthetal, Germany
e-mail: mschulze@dife.de

E. H. J. M. Jansen
Laboratory for Health Protection Research, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

A. Fritsche
Department of Internal Medicine, Division of Endocrinology, Diabetology, Nephrology, Vascular Disease, and Clinical Chemistry, University of Tübingen, Tübingen, Germany

F. Döring
Department of Molecular Nutrition, Institute of Human Nutrition and Food Science, Christian-Albrechts-University, Kiel, Germany

Introduction

Epidemiologic studies suggest beneficial effects of polyunsaturated fatty acid (PUFA) intake on incidence of type 2

diabetes [41] and cardiovascular diseases (CVD) [10]. However, the role of individual PUFA has not been sufficiently elucidated and mechanisms mediating these effects are not completely understood. Investigating PUFA in relation to disease risk markers can provide insight into these issues. For this purpose, blood PUFA composition is of particular interest as it may represent a more accurate indicator of intake than self-reported dietary data [18]. In addition, it also reflects endogenous PUFA metabolism [18], thus providing a more proximal and integrated measure of exposure.

C-reactive protein (CRP) is an acute-phase reactant primarily synthesized in hepatocytes [5]. Chronic inflammation, involving acute-phase reactants, has been hypothesized to promote insulin resistance and atherogenesis, and modestly elevated CRP concentrations predicted higher risk of diabetes [23] and coronary heart disease [20]. Adiponectin, a hormone solely secreted by adipocytes [19], inhibits inflammation, improves insulin sensitivity, and exhibits further atheroprotective effects [19]. Circulating adiponectin was consistently inversely associated with diabetes incidence [24] and tended to be inversely related to risk of coronary heart disease [44].

A limited number of observational studies have examined the relations of blood PUFA to circulating CRP [11, 13, 28, 36, 37, 49] or adiponectin [12, 15, 28, 40]. PUFA from the n-3 series, particularly the long-chain (LC) fatty acids (FA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been included in all of these studies and have been found to be inconsistently associated with both CRP and adiponectin. Little is known about the potential importance of n-6 PUFA, preferentially consumed and metabolized in populations adhering to a Western diet, as modulators of CRP [13, 36, 37] or adiponectin [12].

It has been proposed that the transcription factor peroxisome proliferator-activated receptor (PPAR) γ may partly mediate physiological responses to PUFA, which are biological ligands [4, 50]. Modification of the associations of mainly dietary PUFA with body fat, markers of lipid and glucose metabolism, and risk of myocardial infarction by the Pro12Ala polymorphism has been demonstrated [14, 25, 26, 43, 56]. To our knowledge, interaction between PUFA and Pro12Ala on inflammatory markers or adiponectin has not been explored to date.

The objective of our study was to investigate individual erythrocyte n-6 and n-3 PUFA in relation to plasma CRP and adiponectin in a middle-aged German population. In addition, we evaluated whether the Pro12Ala polymorphism in PPARG2 modified these associations.

Methods

Study design and participants

We conducted a cross-sectional analysis among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study [2] that includes 16,644 women mainly aged 35–64 years and 10,904 men mainly aged 40–64 years recruited from the general population of the city of Potsdam, Germany, and surrounding municipalities from 1994 to 1998. At baseline, data on diet, lifestyle, and medication were obtained by questionnaires and interviews. Anthropometric data and blood samples were collected in physical examinations. All participants gave written informed consent. The Ethical Committee of the State of Brandenburg, Germany, approved the study protocol.

A random sample of 2,500 persons out of the EPIC-Potsdam participants was drawn in order to analyze biochemical and genetic markers. From this subsample, we excluded persons with insufficient blood ($n = 163$), missing data on study variables ($n = 123$), obviously erroneous data on risk markers ($n = 12$), CRP > 10 mg/L indicating acute inflammation ($n = 77$), unreliable data on erythrocyte FA ($n = 143$), and women who were pregnant or nursing ($n = 2$), leaving a total of 1,980 persons for the present analysis. The analytic sample was comparable to the total cohort with regard to sociodemographic, lifestyle, and anthropometric characteristics at baseline (data not shown).

Measurement of CRP, adiponectin, and erythrocyte membrane phospholipid PUFA

Peripheral venous blood samples were drawn from mainly nonfasting participants. Plasma, buffy coat, and erythrocytes were separated by centrifugation (1,000g, 10 min, 4 °C) and aliquots stored at −80 °C. Plasma CRP concentrations were measured in 2007 using a high sensitivity latex-enhanced immunoturbidimetric assay on the automatic ADIVA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany). We treated CRP values below the detection limit of 0.1 mg/L as equal to 0.1 mg/L. Plasma total adiponectin concentrations were determined in 2008 by an ELISA (Linco Research, St. Charles, MO, USA). Plasma samples had been thawed twice before adiponectin measurement. The FA composition of erythrocyte membrane phospholipids was determined from February to June 2008 with methods described in detail elsewhere [22]. Briefly, FA methyl esters were separated on a GC-3900 gas chromatograph (Varian Associates, Middelburg, The Netherlands) equipped with a 100 m × 0.25 mm ID

WCOT-fused silica capillary column and a flame ionization detector with separation of FAME peaks based on mixed FAME standards (Sigma–Aldrich, St. Louis, USA). We optimized the temperature profile in such a way that all peaks of interest were separated. In a typical standard mixture, retention times were 15.99 min for 20:2n-6, 16.79 min for 20:3n-6, 13.22 min for 18:3n-6, and 13.81 min for 18:3n-3. The main abundant conjugated linoleic acid (rumenic acid) has a retention time of 14.59 min and did not interfere. We have used no internal standard, because we discovered that the deuterium-labeled standards were not pure and gave rise to additional peaks in the chromatogram. Individual FA were expressed as a percentage of total FA present in the chromatogram. A total of six n-6 and four n-3 PUFA were separated by this method. Intra-assay CV was <5% for most PUFA, except for eicosadienoic acid (EDA, 7.7%), α-linolenic acid (ALA, 10.2%), and γ-linolenic acid (GLA, 18.7%).

Measurement of other variables

For genetic analyses, DNA was extracted from buffy coat samples. Participants were genotyped for the Pro12Ala polymorphism in PPARG2 using the TaqMan technology (Applied Biosystems, Foster City, CA, USA) on 384-well plates. The reproducibility of the genotyping method was ≥99.5%. The allele frequencies in the present sample were in Hardy–Weinberg equilibrium ($\chi^2 = 0.51$, $p > 0.05$).

Participants were asked for current and past tobacco consumption, average daily alcohol intake and time per week spent on sports activities in the past year, as well as for use of medication and nutritional supplements in the preceding month. Habitual food consumption in the past year was assessed by means of a semiquantitative food frequency questionnaire (FFQ). Energy and nutrient intake were calculated using the German Food Code and Nutrient Data Base. Anthropometric measurements were performed on participants wearing only light underwear and without shoes. Waist circumference was measured midway between the lower rib margin and the superior anterior iliac spine with a nonstretching tape applied horizontally.

Statistical analysis

We characterized study participants with regard to demographic, lifestyle, anthropometric, and genetic features as well as to outcomes and exposures by computing arithmetic means and SD, or medians and quartile ranges (25th and 75th percentiles) in case of asymmetrical distributions, for quantitative variables. We calculated percentages and numbers for categorical variables.

We performed multivariable linear regression analysis in order to investigate the relations of erythrocyte PUFA to plasma CRP and adiponectin. We stratified our models by sex as the risk marker distributions considerably differed between women and men in our study. We used the $\log_e$ transformed distributions of CRP and adiponectin to stabilize the variances and to normalize the distributions of the dependent variables. We modeled individual n-6 and n-3 PUFA proportions categorized into sex-specific quintiles to account for nonlinear relations with the outcomes. We estimated geometric means and 95% CI of CRP and adiponectin by PUFA quintiles and tested for statistical significance of linear trends across quintiles by modeling the median value of the PUFA within each quintile as a quantitative variable. In a first model, we controlled for age (<45, 45–49, 50–54, 55–59, ≥60 years) only. In a second model, we further adjusted for smoking status (never, past, current <20 units/days, current ≥20 units/days), alcohol intake (0, >0–<15, 15–<30, 30–<40, ≥40 g/days), sports activities (0, >0–2, >2–4, >4–6, >6 h/week), and hormone use (none, oral contraceptive, hormone replacement therapy [HRT], in women only). PUFA have been shown to affect body fat deposition at multiple critical points [30, 34, 47], and body fat is a key determinant of circulating inflammatory markers and adiponectin [16]. Thus, we considered body fat as a potential intermediate in the causal pathways from PUFA to CRP and adiponectin and included waist circumference in a third model. In a sensitivity analysis, we further entered dietary factors related to the outcomes in some studies, namely cholesterol (mg/days, quintiles), total fiber (g/days, quintiles), glycemic index (quintiles), and vitamin E supplements (no, yes) in model 2. In another sensitivity analysis, we excluded persons with type 2 diabetes or CVD to partly account for alterations in blood PUFA resulting from inflammation or hypoadiponectinemia.

We conducted stratified analyses to explore whether the associations of erythrocyte PUFA with CRP and adiponectin varied by PPARG2 genotype. We compared homozygous carriers of the Pro12 allele to carriers of the Ala12 allele. We a priori restricted exposures to LC PUFA (20 or 22 carbons, except for the intermediate EDA), which may be most relevant for modulation of inflammation and metabolic function [3, 8]. Also, we considered linoleic acid (LA) given the strong evidence indicating beneficial effects of LA on metabolic mediators and disease risk [10, 41]. We tested for interaction by evaluating statistical significance of cross-product terms of the respective PUFA (as a quantitative variable) and genotype included in the multivariable models.

We performed the statistical analysis with the SAS software, release 9.2 (SAS Institute Inc., Cary, NC, USA). All statistical tests were two tailed. We considered $p < 0.05$ being statistically significant.

Results

The mean age was 49 years in women ($n = 1{,}222$) and 52 years in men ($n = 758$) (Table 1). Plasma concentrations of both CRP and adiponectin were considerably higher among women compared to men. Erythrocyte PUFA contents were largely comparable between women and men, except for lower GLA (C18:3n-6) and slightly higher DHA (C22:6n-3) in women. Arachidonic acid (AA, C20:4n-6) and LA (C18:2n-6) were most abundant with proportions greater than 10%. Dihomo-γ-linolenic acid (DGLA, C20:3n-6), docosatetraenoic acid (DTA, C22:4n-6), docosapentaenoic acid (DPA, C22:5n-3), and DHA were present in proportions of 1–5%. GLA, EDA (C20:2n-6), ALA (C18:3n-3), and EPA (C20:5n-3) each accounted for less than 1% of total FA.

Among women, CRP significantly decreased across increasing quintiles of erythrocyte LA and DPA, whereas CRP significantly increased across quintiles of GLA, EDA, and DGLA in models adjusted for age (data not shown). These PUFA remained related to CRP after additional control for lifestyle variables (Table 2) (geometric mean of CRP [mg/L], quintile 1, quintile 5, p for trend, LA: 0.95, 0.61, $p = 0.0001$, GLA: 0.63, 0.92, $p = 0.0002$, EDA: 0.75, 0.93, $p = 0.06$, DGLA: 0.55, 1.07, $p < 0.0001$, DPA: 0.86, 0.61, $p = 0.002$). After further adjustment for waist circumference, these associations were mostly attenuated but still significant or borderline significant. Among men, CRP significantly decreased across quintiles of LA, whereas CRP significantly increased across quintiles of GLA and DGLA in age-adjusted models. LA, GLA, and DGLA remained associated with CRP by adjustment for lifestyle factors (Table 2) (geometric mean of CRP [mg/L], quintile 1, quintile 5, p for trend, LA: 0.67, 0.51, $p = 0.01$, GLA: 0.55, 0.70, $p = 0.08$, DGLA: 0.52, 0.76, $p = 0.0003$). Including waist circumference in the models, only DGLA remained related to CRP albeit weaker.

In women, adiponectin significantly increased across quintiles of LA, whereas adiponectin significantly decreased across quintiles of GLA and DGLA after adjustment for age (data not shown). Further control for lifestyle variables did not appreciably change these associations (Table 3) (geometric mean of adiponectin [mg/L], quintile 1, quintile 5, p for trend, LA: 7.9, 9.1, $p = 0.0005$,

Table 1 Characteristics of persons of a subsample of the EPIC-Potsdam cohort by sex [For quantitative variables, values are arithmetic means ± SD or medians (25th–75th percentile)]

Characteristic	Women ($n = 1{,}222$)	Men ($n = 758$)
General characteristics		
Age (years)	48.6 ± 9.2	51.9 ± 8.2
Smoking status (% (n))		
Never	59.3 (725)	29.4 (223)
Past	22.6 (276)	44.1 (334)
Current (<20 units/days)	14.7 (180)	16.1 (122)
Current (≥20 units/days)	3.4 (41)	10.4 (79)
Alcohol intake (g/days)	5.0 (1.7–10.5)	17.3 (7.5–32.3)
Sports activities (h/week)	0 (0–1.5)	0 (0–1.5)
Hormone use (% (n))		
Oral contraceptive	14.6 (179)	–
HRT	23.6 (288)	–
BMI (kg/m^2)	25.5 ± 4.4	26.7 ± 3.6
Waist circumference (cm)	80.4 ± 11.3	93.9 ± 10.1
CRP (mg/L)	0.77 (0.22–2.09)	0.55 (0.22–1.43)
Adiponectin (mg/L)	8.66 (6.37–11.45)	5.67 (4.21–7.51)
Erythrocyte membrane phospholipid n-6 and n-3 PUFA (% of total FA)		
LA (C18:2n-6)	10.75 (9.86–11.63)	10.53 (9.57–11.36)
GLA (C18:3n-6)	0.050 (0.037–0.068)	0.061 (0.045–0.077)
EDA (C20:2n-6)	0.26 (0.23–0.28)	0.25 (0.22–0.27)
DGLA (C20:3n-6)	1.48 (1.29–1.71)	1.46 (1.27–1.65)
AA (C20:4n-6)	13.21 (12.02–14.16)	12.94 (11.69–13.85)
DTA (C22:4n-6)	2.72 (2.33–3.08)	2.59 (2.15–2.95)
ALA (C18:3n-3)	0.15 (0.12–0.18)	0.15 (0.12–0.17)
EPA (C20:5n-3)	0.75 (0.57–0.94)	0.78 (0.59–0.99)
DPA (C22:5n-3)	2.30 (2.00–2.57)	2.40 (2.08–2.66)
DHA (C22:6n-3)	4.85 (4.08–5.60)	4.55 (3.65–5.21)

Table 2 Geometric means (95% CI) of plasma CRP concentrations (mg/L) by quintiles of erythrocyte PUFA proportions in a subsample of the EPIC-Potsdam cohort

	Women (n = 1,222)						Men (n = 758)					
	Quintile of erythrocyte PUFA, % of total FA					p for trend	Quintile of erythrocyte PUFA, % of total FA					p for trend
	1	2	3	4	5		1	2	3	4	5	
LA (C18:2n-6)												
Median[1]	9.18	10.03	10.75	11.44	12.43		8.94	9.79	10.52	11.15	12.18	
Model 1	0.95 (0.81–1.11)	0.74 (0.63–0.86)[a]	0.81 (0.69–0.94)	0.66 (0.57–0.77)[b]	0.61 (0.53–0.72)[c]	0.0001	0.67 (0.55–0.81)	0.66 (0.55–0.80)	0.67 (0.56–0.81)	0.49 (0.40–0.59)[a]	0.51 (0.42–0.62)	0.01
Model 2	0.88 (0.77–1.02)	0.73 (0.64–0.84)	0.79 (0.69–0.91)	0.67 (0.59–0.77)[b]	0.67 (0.58–0.77)[b]	0.005	0.62 (0.52–0.75)	0.63 (0.52–0.75)	0.67 (0.56–0.80)	0.50 (0.42–0.60)	0.56 (0.46–0.67)	0.18
GLA (C18:3n-6)												
Median	0.026	0.040	0.050	0.064	0.088		0.031	0.048	0.061	0.074	0.094	
Model 1	0.63 (0.54–0.74)	0.69 (0.60–0.81)	0.67 (0.57–0.78)	0.85 (0.73–1.00)[b]	0.92 (0.78–1.07)[b]	0.0002	0.55 (0.45–0.66)	0.60 (0.50–0.73)	0.50 (0.41–0.60)	0.64 (0.53–0.77)	0.70 (0.58–0.84)	0.08
Model 2	0.70 (0.61–0.80)	0.72 (0.63–0.83)	0.66 (0.58–0.76)	0.85 (0.74–0.97)	0.81 (0.70–0.93)	0.05	0.58 (0.48–0.69)	0.64 (0.53–0.76)	0.50 (0.42–0.60)	0.63 (0.52–0.75)	0.63 (0.52–0.76)	0.54
EDA (C20:2n-6)												
Median	0.21	0.24	0.26	0.28	0.31		0.21	0.23	0.25	0.27	0.30	
Model 1	0.75 (0.64–0.88)	0.69 (0.59–0.80)	0.67 (0.58–0.79)	0.70 (0.60–0.82)	0.93 (0.79–1.09)	0.06	0.58 (0.48–0.70)	0.63 (0.52–0.77)	0.62 (0.51–0.75)	0.52 (0.43–0.63)	0.61 (0.51–0.74)	0.82
Model 2	0.77 (0.66–0.88)	0.69 (0.60–0.79)	0.68 (0.59–0.78)	0.69 (0.60–0.79)	0.93 (0.81–1.07)	0.05	0.59 (0.50–0.71)	0.63 (0.53–0.76)	0.59 (0.49–0.71)	0.54 (0.45–0.65)	0.61 (0.51–0.73)	0.78
DGLA (C20:3n-6)												
Median	1.12	1.33	1.48	1.66	1.90		1.09	1.31	1.46	1.60	1.86	
Model 1	0.55 (0.47–0.64)	0.59 (0.51–0.68)	0.72 (0.62–0.84)[a]	0.92 (0.79–1.07)[d]	1.07 (0.92–1.24)[d]	<0.0001	0.52 (0.43–0.62)	0.47 (0.39–0.57)	0.59 (0.49–0.72)	0.68 (0.56–0.82)[a]	0.76 (0.63–0.91)[b]	0.0003
Model 2	0.63 (0.55–0.72)	0.65 (0.57–0.75)	0.70 (0.61–0.81)	0.83 (0.73–0.96)[b]	0.96 (0.83–1.10)[d]	<0.0001	0.55 (0.46–0.66)	0.48 (0.40–0.58)	0.61 (0.51–0.73)	0.64 (0.54–0.77)	0.71 (0.59–0.85)	0.009
AA (C20:4n-6)												
Median	10.03	12.30	13.21	13.95	14.97		8.71	11.94	12.94	13.65	14.68	
Model 1	0.78 (0.67–0.91)	0.68 (0.58–0.79)	0.77 (0.66–0.89)	0.73 (0.63–0.86)	0.77 (0.66–0.90)	0.94	0.59 (0.49–0.72)	0.59 (0.49–0.71)	0.53 (0.44–0.64)	0.61 (0.50–0.73)	0.66 (0.54–0.80)	0.64
Model 2	0.79 (0.69–0.90)	0.67 (0.59–0.77)	0.74 (0.65–0.85)	0.74 (0.65–0.85)	0.78 (0.68–0.90)	0.98	0.60 (0.50–0.72)	0.57 (0.48–0.69)	0.52 (0.43–0.62)	0.63 (0.52–0.75)	0.66 (0.55–0.79)	0.66
DTA (C22:4n-6)												
Median	1.79	2.44	2.72	3.01	3.44		1.61	2.23	2.59	2.88	3.21	
Model 1	0.74 (0.63–0.86)	0.71 (0.61–0.83)	0.81 (0.69–0.94)	0.68 (0.58–0.79)	0.79 (0.68–0.93)	0.69	0.55 (0.45–0.66)	0.66 (0.54–0.79)	0.55 (0.45–0.66)	0.56 (0.46–0.67)	0.67 (0.55–0.81)	0.39

Table 2 continued

	Women (n = 1,222)						Men (n = 758)					
	Quintile of erythrocyte PUFA, % of total FA					p for trend	Quintile of erythrocyte PUFA, % of total FA					p for trend
	1	2	3	4	5		1	2	3	4	5	
Model 2	0.75 (0.65–0.86)	0.70 (0.61–0.80)	0.79 (0.69–0.90)	0.71 (0.62–0.81)	0.78 (0.68–0.90)	0.68	0.56 (0.47–0.68)	0.65 (0.54–0.78)	0.53 (0.44–0.63)	0.58 (0.49–0.70)	0.66 (0.55–0.79)	0.52
ALA (C18:3n-3)												
Median	0.11	0.13	0.15	0.18	0.22		0.10	0.13	0.15	0.17	0.22	
Model 1	0.80 (0.69–0.94)	0.71 (0.61–0.82)	0.83 (0.71–0.97)	0.64 (0.55–0.75)[a]	0.75 (0.65–0.88)	0.44	0.59 (0.49–0.71)	0.52 (0.43–0.62)	0.69 (0.57–0.83)	0.67 (0.55–0.81)	0.52 (0.43–0.63)	0.69
Model 2	0.77 (0.67–0.88)	0.70 (0.61–0.80)	0.84 (0.73–0.96)	0.69 (0.60–0.80)	0.74 (0.64–0.85)	0.67	0.58 (0.48–0.69)	0.52 (0.43–0.62)	0.68 (0.56–0.81)	0.67 (0.55–0.80)	0.54 (0.45–0.65)	0.94
EPA (C20:5n-3)												
Median	0.42	0.61	0.75	0.89	1.18		0.44	0.62	0.78	0.94	1.24	
Model 1	0.69 (0.59–0.81)	0.79 (0.67–0.92)	0.75 (0.64–0.87)	0.74 (0.64–0.87)	0.75 (0.64–0.88)	0.65	0.62 (0.51–0.75)	0.59 (0.49–0.72)	0.53 (0.44–0.65)	0.55 (0.45–0.66)	0.69 (0.57–0.83)	0.51
Model 2	0.73 (0.64–0.84)	0.79 (0.69–0.91)	0.71 (0.62–0.82)	0.73 (0.63–0.83)	0.76 (0.66–0.88)	0.91	0.65 (0.54–0.78)	0.60 (0.50–0.72)	0.53 (0.44–0.63)	0.56 (0.47–0.68)	0.64 (0.53–0.77)	0.93
DPA (C22:5n-3)												
Median	1.54	2.09	2.30	2.51	2.80		1.43	2.16	2.40	2.59	2.88	
Model 1	0.86 (0.74–1.01)	0.91 (0.78–1.06)	0.63 (0.54–0.73)[b]	0.76 (0.65–0.89)	0.61 (0.52–0.71)[b]	0.002	0.57 (0.47–0.70)	0.61 (0.50–0.74)	0.68 (0.56–0.82)	0.53 (0.43–0.63)	0.59 (0.49–0.72)	0.98
Model 2	0.87 (0.75–1.00)	0.81 (0.70–0.93)	0.66 (0.58–0.76)[b]	0.72 (0.63–0.83)	0.68 (0.59–0.79)[a]	0.01	0.58 (0.48–0.70)	0.58 (0.49–0.70)	0.69 (0.57–0.82)	0.53 (0.44–0.64)	0.59 (0.49–0.71)	0.99
DHA (C22:6n-3)												
Median	2.91	4.26	4.85	5.41	6.27		2.43	3.81	4.55	5.05	6.02	
Model 1	0.72 (0.62–0.84)	0.72 (0.62–0.85)	0.78 (0.67–0.91)	0.72 (0.62–0.84)	0.78 (0.67–0.91)	0.49	0.67 (0.55–0.81)	0.50 (0.41–0.60)[a]	0.54 (0.45–0.66)	0.62 (0.51–0.74)	0.66 (0.55–0.80)	0.87
Model 2	0.75 (0.65–0.86)	0.69 (0.60–0.80)	0.77 (0.67–0.88)	0.73 (0.63–0.84)	0.79 (0.69–0.91)	0.54	0.69 (0.58–0.83)	0.51 (0.43–0.62)[a]	0.54 (0.45–0.65)	0.60 (0.50–0.73)	0.63 (0.52–0.75)	0.59

Model 1 adjusted for age (<45, 45–49, 50–54, 55–59, ≥60 years), smoking status (never, past, current <20 units/days, current ≥20 units/days), alcohol intake (0, >0–<15, 15–<30, 30–<40, ≥40 g/days), sports activities (0, >0–2, >2–4, >4–6, >6 h/week), and hormone use (none, oral contraceptive, HRT; women only). Model 2 additionally adjusted for waist circumference (cm, quintiles)

[1] Values are medians of the respective PUFA (% of total FA)

[a–d] Geometric mean significantly different from quintile 1: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, [d] $p < 0.0001$

Table 3 Geometric means (95% CI) of plasma adiponectin concentrations (mg/L) by quintiles of erythrocyte PUFA proportions in a subsample of the EPIC-Potsdam cohort

	Women (n = 1,222)						Men (n = 758)					
	Quintile of erythrocyte PUFA (% of total FA)					p for trend	Quintile of erythrocyte PUFA (% of total FA)					p for trend
	1	2	3	4	5		1	2	3	4	5	
LA (C18:2n-6)												
Median[1]	9.18	10.03	10.75	11.44	12.43		8.94	9.79	10.52	11.15	12.18	
Model 1	7.86 (7.43–8.32)	8.20 (7.76–8.67)	8.37 (7.92–8.85)	8.33 (7.87–8.80)	9.11 (8.62–9.64)[c]	0.0005	5.33 (4.94–5.75)	5.15 (4.78–5.55)	5.68 (5.27–6.12)	5.60 (5.20–6.04)	6.08 (5.64–6.56)[a]	0.005
Model 2	8.00 (7.58–8.45)	8.24 (7.81–8.69)	8.41 (7.97–8.87)	8.28 (7.85–8.74)	8.93 (8.46–9.42)[b]	0.009	5.43 (5.04–5.85)	5.24 (4.87–5.64)	5.68 (5.28–6.11)	5.58 (5.18–6.00)	5.90 (5.48–6.36)	0.06
GLA (C18:3n-6)												
Median	0.026	0.040	0.050	0.064	0.088		0.031	0.048	0.061	0.074	0.094	
Model 1	8.65 (8.18–9.15)	8.76 (8.29–9.27)	8.45 (7.99–8.93)	8.04 (7.60–8.50)	7.96 (7.52–8.43)[a]	0.008	5.83 (5.40–6.29)	5.83 (5.41–6.28)	5.31 (4.93–5.73)	5.47 (5.08–5.90)	5.39 (4.99–5.81)	0.08
Model 2	8.44 (7.99–8.91)	8.66 (8.21–9.14)	8.47 (8.03–8.94)	8.06 (7.63–8.50)	8.21 (7.77–8.67)	0.19	5.71 (5.30–6.15)	5.73 (5.32–6.16)	5.33 (4.95–5.74)	5.47 (5.08–5.89)	5.58 (5.18–6.01)	0.50
EDA (C20:2n-6)												
Median	0.21	0.24	0.26	0.28	0.31		0.21	0.23	0.25	0.27	0.30	
Model 1	8.07 (7.62–8.54)	8.50 (8.03–8.99)	8.31 (7.86–8.79)	8.77 (8.29–9.28)[a]	8.20 (7.74–8.69)	0.62	5.39 (5.00–5.81)	5.48 (5.09–5.91)	5.48 (5.08–5.91)	5.69 (5.28–6.13)	5.77 (5.36–6.22)	0.15
Model 2	8.05 (7.62–8.51)	8.49 (8.05–8.97)	8.32 (7.88–8.77)	8.78 (8.32–9.27)[a]	8.20 (7.76–8.67)	0.55	5.32 (4.94–5.72)	5.55 (5.16–5.97)	5.55 (5.15–5.97)	5.58 (5.19–6.01)	5.82 (5.41–6.26)	0.11
DGLA (C20:3n-6)												
Median	1.12	1.33	1.48	1.66	1.90		1.09	1.31	1.46	1.60	1.86	
Model 1	9.16 (8.67–9.69)	8.63 (8.16–9.12)	8.27 (7.83–8.75)[a]	7.95 (7.51–8.40)[c]	7.89 (7.46–8.34)[c]	<0.0001	5.88 (5.46–6.34)	5.76 (5.35–6.21)	5.22 (4.85–5.63)[a]	5.58 (5.18–6.01)	5.38 (4.99–5.80)	0.08
Model 2	8.89 (8.42–9.39)	8.42 (7.98–8.88)	8.32 (7.88–8.77)	8.15 (7.72–8.61)[a]	8.08 (7.66–8.53)[a]	0.01	5.75 (5.34–6.18)	5.71 (5.30–6.13)	5.22 (4.85–5.61)	5.67 (5.27–6.10)	5.48 (5.09–5.90)	0.39
AA (C20:4n-6)												
Median	10.03	12.30	13.21	13.95	14.97		8.71	11.94	12.94	13.65	14.68	
Model 1	8.55 (8.08–9.04)	8.59 (8.12–9.09)	8.45 (7.99–8.93)	7.99 (7.55–8.45)	8.28 (7.82–8.75)	0.18	5.51 (5.11–5.94)	5.67 (5.26–6.11)	5.62 (5.22–6.06)	5.48 (5.09–5.91)	5.51 (5.11–5.95)	0.96
Model 2	8.53 (8.08–9.00)	8.62 (8.17–9.10)	8.51 (8.07–8.98)	7.96 (7.54–8.40)	8.23 (7.80–8.68)	0.14	5.49 (5.10–5.90)	5.71 (5.31–6.15)	5.67 (5.27–6.10)	5.41 (5.03–5.82)	5.53 (5.13–5.95)	
DTA (C22:4n-6)												
Median	1.79	2.44	2.72	3.01	3.44		1.61	2.23	2.59	2.88	3.21	
Model 1	8.49 (8.03–8.98)	8.51 (8.05–9.01)	8.22 (7.77–8.69)	8.24 (7.79–8.72)	8.37 (7.91–8.86)	0.53	5.99 (5.56–6.46)	5.83 (5.41–6.29)	5.50 (5.10–5.93)	5.34 (4.96–5.76)[a]	5.18 (4.80–5.58)[b]	0.002

Table 3 continued

	Women (n = 1,222)						Men (n = 758)					
	Quintile of erythrocyte PUFA (% of total FA)					p for trend	Quintile of erythrocyte PUFA (% of total FA)					p for trend
	1	2	3	4	5		1	2	3	4	5	
Model 2	8.46 (8.02–8.93)	8.54 (8.09–9.01)	8.29 (7.85–8.74)	8.16 (7.73–8.61)	8.38 (7.94–8.86)	0.52	5.93 (5.51–6.38)	5.85 (5.43–6.29)	5.60 (5.20–6.02)	5.28 (4.91–5.68)[a]	5.20 (4.83–5.59)[a]	0.003
ALA (C18:3n-3)												
Median	0.11	0.13	0.15	0.18	0.22		0.10	0.13	0.15	0.17	0.22	
Model 1	7.94 (7.51–8.40)	8.64 (8.17–9.14)[a]	8.29 (7.84–8.76)	8.63 (8.16–9.13)[a]	8.35 (7.89–8.83)	0.35	5.30 (4.92–5.71)	5.25 (4.87–5.65)	5.47 (5.07–5.89)	5.68 (5.27–6.13)	6.15 (5.71–6.63)[b]	0.001
Model 2	8.05 (7.62–8.49)	8.67 (8.21–9.14)	8.28 (7.84–8.73)	8.47 (8.03–8.94)	8.39 (7.95–8.85)	0.52	5.32 (4.94–5.72)	5.27 (4.90–5.67)	5.50 (5.11–5.92)	5.68 (5.27–6.11)	6.07 (5.64–6.53)[a]	0.003
EPA (C20:5n-3)												
Median	0.42	0.61	0.75	0.89	1.18		0.44	0.62	0.78	0.94	1.24	
Model 1	8.79 (8.30–9.32)	8.16 (7.72–8.64)	8.09 (7.65–8.55)[a]	8.20 (7.75–8.68)	8.60 (8.12–9.12)	0.83	5.50 (5.11–5.93)	5.37 (4.98–5.79)	5.67 (5.26–6.11)	5.88 (5.45–6.33)	5.40 (5.00–5.82)	0.87
Model 2	8.71 (8.25–9.21)	8.15 (7.73–8.61)	8.17 (7.75–8.62)	8.24 (7.81–8.70)	8.56 (8.10–9.05)	0.91	5.41 (5.02–5.82)	5.37 (4.99–5.78)	5.70 (5.30–6.13)	5.81 (5.41–6.25)	5.52 (5.13–5.95)	0.41
DPA (C22:5n-3)												
Median	1.54	2.09	2.30	2.51	2.80		1.43	2.16	2.40	2.59	2.88	
Model 1	8.41 (7.94–8.90)	8.25 (7.80–8.74)	8.31 (7.86–8.79)	8.32 (7.86–8.81)	8.54 (8.07–9.04)	0.77	5.64 (5.23–6.08)	5.42 (5.03–5.84)	5.73 (5.32–6.18)	5.89 (5.46–6.34)	5.15 (4.78–5.56)	0.44
Model 2	8.42 (7.97–8.89)	8.50 (8.05–8.98)	8.21 (7.78–8.66)	8.42 (7.97–8.89)	8.29 (7.85–8.76)	0.69	5.60 (5.20–6.03)	5.46 (5.07–5.87)	5.74 (5.34–6.18)	5.88 (5.46–6.32)	5.16 (4.79–5.55)	0.50
DHA (C22:6n-3)												
Median	2.91	4.26	4.85	5.41	6.27		2.43	3.81	4.55	5.05	6.02	
Model 1	8.59 (8.12–9.08)	8.66 (8.19–9.16)	8.44 (7.98–8.92)	8.03 (7.60–8.49)	8.13 (7.68–8.60)	0.06	5.67 (5.26–6.11)	5.82 (5.39–6.27)	5.44 (5.04–5.86)	5.77 (5.35–6.22)	5.14 (4.76–5.54)	0.10
Model 2	8.53 (8.08–9.00)	8.77 (8.31–9.26)	8.47 (8.03–8.94)	7.99 (7.58–8.44)	8.09 (7.66–8.54)	0.05	5.58 (5.19–6.01)	5.76 (5.35–6.20)	5.43 (5.05–5.84)	5.83 (5.42–6.27)	5.22 (4.85–5.62)	0.32

Model 1 adjusted for age (<45, 45–49, 50–54, 55–59, ≥60 years), smoking status (never, past, current <20 units/days, current ≥20 units/days), alcohol intake (0, >0–<15, 15–<30, 30–<40, ≥40 g/days), sports activities (0, >0–2, >2–4, >4–6, >6 h/week), and hormone use (none, oral contraceptive, HRT; women only). Model 2 additionally adjusted for waist circumference (cm, quintiles)

[1] Values are medians of the respective PUFA (% of total FA)

[a–c] Geometric mean significantly different from quintile 1: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$

GLA: 8.6, 8.0, $p = 0.008$, DGLA: 9.2, 7.9, $p < 0.0001$). Adjustment for waist circumference attenuated these relations, with GLA being no longer associated with adiponectin. In men, adiponectin significantly increased across quintiles of LA and ALA, whereas it significantly decreased across quintiles of DTA in age-adjusted models. DGLA also tended to be inversely associated with adiponectin ($p = 0.08$). Control for lifestyle factors did not greatly alter these associations (Table 3) and GLA now also tended to be inversely related to adiponectin (geometric mean of adiponectin [mg/L], quintile 1, quintile 5, p for trend, LA: 5.3, 6.1, $p = 0.005$, GLA: 5.8, 5.4, $p = 0.08$, DGLA: 5.9, 5.4, $p = 0.08$, DTA: 6.0, 5.2, $p = 0.002$, ALA: 5.3, 6.1, $p = 0.001$). By further adjustment for waist circumference, LA, DTA, and ALA remained associated with adiponectin, while the relations of GLA and DGLA to adiponectin disappeared.

After adjustment for age and lifestyle, further control for diet and vitamin E supplementation did not appreciably affect the observed relations (data not shown). Excluding persons with diabetes or CVD also did not substantially change the estimates (data not shown), with one exception. In women, AA was now significantly inversely related to adiponectin (p for trend = 0.04).

The frequencies of the Ala12 allele of PPARG2 were 16% in women and 14% in men. Twenty-nine percent of women and 26% of men carried the Ala12 allele. In women, the associations of AA and DTA with CRP varied by PPARG2 genotype after control for age and lifestyle. AA was inversely associated with CRP in Ala12 carriers, whereas no clear association was seen in Pro12 homozygotes (Fig. 1a, p for interaction = 0.04). DTA was also inversely related to CRP in Ala12 carriers, while a positive trend was apparent in noncarriers (Fig. 1b), though the interaction was only borderline significant ($p = 0.08$). These differences were attenuated and lost significance by further adjustment for waist circumference ($p = 0.15$ for AA, $p = 0.20$ for DTA). The association of DTA with adiponectin also differed between Ala12 variants in women. DTA was positively associated with adiponectin in Ala12 carriers, whereas a slight inverse trend was seen in noncarriers adjusting for age and lifestyle (Fig. 1c, $p = 0.02$). Albeit attenuated, this interaction remained significant by adjustment for waist circumference ($p = 0.04$). Also, Pro12Ala appeared to interact with DGLA on adiponectin in women (inverse association only in Pro12 homozygotes), although not significantly ($p = 0.13$, data not graphed). Other LC PUFA or LA did neither strongly nor significantly differ by PPARG2 genotype with regard to their associations to CRP or adiponectin among women (data not shown). In men, the relations under study were largely independent of the Pro12Ala polymorphism (data not shown).

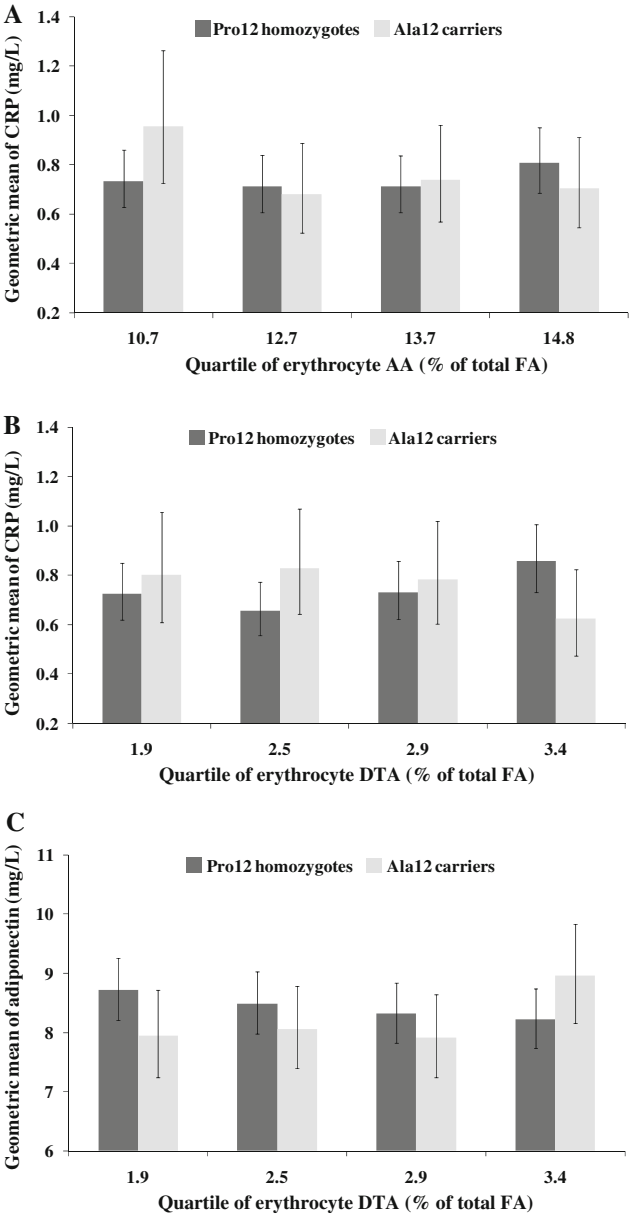

Fig. 1 Geometric means of plasma CRP and adiponectin by quartiles of erythrocyte PUFA and PPARG2 genotype. Only associations with corresponding p values for interaction <0.10 are shown. All of these associations were observed in women. *Error bars* represent 95% CI of estimated geometric means. Medians of PUFA within quartiles are given on the x-axis. Models were adjusted for age (y), smoking status (never, past, current), alcohol intake (0, >0–<15, 15–<30, ≥30 g/days), sports activities (0, >0–2, >2–4, >4 h/week), and hormone use (none, oral contraceptive, HRT). p for interaction: 0.04 (**a**), 0.08 (**b**), 0.02 (**c**). $N = 868$ for Pro12 homozygotes, $n = 354$ for Ala12 carriers

Discussion

In this study, higher erythrocyte LA was related to lower CRP and higher adiponectin, whereas higher GLA and DGLA were related to higher CRP and lower adiponectin. The relations of erythrocyte PUFA to CRP or adiponectin

mostly did neither strongly nor significantly vary by PPARG2 genotype. In women, the Pro12Ala variant appeared to modify the association of AA with CRP, as well as the associations of DTA with CRP and adiponectin.

Blood PUFA composition reflects both PUFA intake and the activities of enzymes for endogenous desaturation, elongation, and oxidation of PUFA (Online Resource 1) and selective mechanisms which, for example, regulate incorporation of fatty acids into membrane phospholipids. Blood LA tends to correlate well with intake [18], but is also substrate of Δ-6 desaturase (D6D). Thus, the associations of erythrocyte LA with CRP and adiponectin may indicate antiinflammatory and adiponectin-enhancing effects of higher LA intake and/or lower D6D activity. Our findings are in line with two prior investigations [36, 37], although another analysis found no association with CRP [13]. So far, studies on LA in relation to adiponectin are scarce and, in contrast to our data, provided nonsignificant results [7, 12]. Dietary intakes of n-6 HUFA, except for AA, are negligible and their blood contents mainly derive from hepatic desaturation and elongation of LA. Higher GLA (D6D product) and DGLA (D6D product, Δ-5 desaturase [D5D] substrate) contents may indicate higher D6D activity, lower D5D activity, or both. Particularly DGLA and to a lesser extent GLA were unfavorably associated with CRP and adiponectin. Similar associations were observed in previous studies, but were mainly due to associated lifestyle characteristics and BMI [12, 36, 37]. Moreover, a higher erythrocyte AA/LA ratio, reflecting overall higher D6D/D5D activity, as well as variants in genes encoding these enzymes and associated with higher AA/LA, was related to higher CRP [29]. The associations for DGLA and GLA were somewhat more pronounced among women compared to men, but had similar directions—similar to another previous study [12]. Besides the smaller effect size, lower statistical power may be one explanation for nonsignificant associations among men compared to women. Corroborating our and other cross-sectional data, blood GLA and DGLA, as well as PUFA ratios estimating D6D activity, were directly while blood LA and estimated D5D activity were inversely related to risk of diabetes and the metabolic syndrome in several cohort studies, including EPIC-Potsdam [17, 21, 22, 53, 54].

We also found DPA to be inversely related to CRP in women, in agreement with two other cross-sectional studies, suggesting a role of LC n-3 PUFA in inflammation [42, 49]. Blood DPA largely arises from endogenous interconversion of LC n-3 PUFA, which appears to be more efficient in women than in men [1]. Moreover, we observed a positive relation of ALA to adiponectin in men, unlike few prior investigations reporting no or inverse associations of dietary or blood ALA with adiponectin [12, 15, 32, 35].

Blood PUFA composition may modulate circulating CRP and adiponectin through different pathways. PUFA and their metabolites may decrease proinflammatory cytokines by inhibiting nuclear factor (NF)-κB-dependent gene expression [4, 6]. Furthermore, PUFA and their derivatives are ligands for PPARγ [52] whose activation downregulates cytokine and upregulates adiponectin expression [51]. Specifically, nitrated LA has been shown to decrease IL-6 and TNF-α production by macrophages via inhibition of NF-κB [6] and is a high-affinity ligand for PPARγ [45]. In addition, LA inhibits D6D gene expression, thereby suppressing n-6 HUFA synthesis [31]. Contrary to our observations, GLA and DGLA have also been described as antiinflammatory agents [8] and efficient PPARγ ligands [55]. However, D6D inhibition mitigated inflammation in mice by reducing tissue AA [33] known as a PUFA also acting proinflammatory [3]. LC n-3 PUFA have been demonstrated to inhibit IL-6 and TNF-α production, probably mediated by inhibition of NF-κB and/or activation of PPARγ [4]. Although ALA is only a weak PPARγ ligand [55], it still raised plasma adiponectin in rats [46].

In women, AA and DTA tended to be favorably related to CRP and adiponectin in carriers of the minor Ala12 allele of PPARG2, while DTA tended to be unfavorably related to both outcomes in Pro12 homozygotes. Our observation is partly consistent with prior studies, wherein favorable relations of dietary and plasma PUFA to body fat and markers of glucose and lipid metabolism were restricted to or stronger in Ala12 carriers [14, 25, 26, 56]. Yet there is also evidence to the contrary [43, 56]. However, the population effect of Pro12Ala likely depends on numerous factors such as background diet, obesity, and other genetic variants [48].

We included a broader range of both n-3 and n-6 PUFA in comparison with prior investigations. However, as is common practice, individual PUFA were quantified as percentages of total FA in the chromatogram and thus, they depend on the remaining FA proportions as do their associations with risk markers. This might also be the case for FA not determined in our study. For example, lower DPA and DHA concentrations might be accompanied by higher 22:5n-6 concentrations [9]. Moreover, the single FA determination may not have accounted for intraindividual variation in blood PUFA composition [27], which may have attenuated the estimates of the relations under study. Furthermore, measurement error in determining FAs may be an explanation for a lack of association. We also measured CRP and adiponectin only once. While adiponectin measurement has been shown to be highly reproducible [39], CRP is measured with considerable random error [5]. We adjusted the associations of interest for important potential confounders. However, residual confounding due to variables not included and measurement errors in the

available data may still have biased our estimates. Notably, unlike usually done, we presented two models without and with adjustment for waist circumference as mechanistic studies indicate that body fat might mediate effects of PUFA on CRP and adiponectin [30, 34, 47]. Of particular concern, cross-sectional relations of blood PUFA to CRP and adiponectin may partly reflect changes in FA profiles due to inflammation and hypoadiponectinemia [19, 38].

In conclusion, our findings suggest that erythrocyte PUFA, particularly LA and n-6 HUFA, are related to circulating CRP and adiponectin. They do not indicate that erythrocyte PUFA strongly interact with the PPARG2 Pro12Ala polymorphism on these risk markers. Experimental studies are needed to verify that modification of PUFA intake and/or endogenous PUFA conversion indeed modulates circulating inflammatory markers and adiponectin.

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Conflict of interest The authors declare that they have no conflict of interest.

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