Circulating Polyunsaturated Fatty Acids as Biomarkers for Dietary Intake across Subgroups: The CODAM and Hoorn Studies

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Circulating Polyunsaturated Fatty Acids as Biomarkers for Dietary Intake across Subgroups: The CODAM and Hoorn Studies

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Keywords

Dietary fat · Circulating fatty acids · Biomarker · Polyunsaturated fat

Abstract

Aims: To evaluate whether participant characteristics and way of expressing circulating fatty acids (FA) influence the strengths of associations between self-reported intake and circulating levels of linoleic acid (LA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). **Methods:** Cross-sectional analyses were performed in pooled data from the CODAM ($n = 469$) and Hoorn ($n = 702$) studies. Circulating FA were measured by gas liquid chromatography and expressed as proportions (% of total FA) and concentrations ($\mu\text{g/mL}$). Dietary intakes were calcu-

lated from a validated food frequency questionnaire. Effects of participant characteristics on associations between dietary and circulating FA were calculated using interaction analyses. **Results:** Standardized regression coefficients between dietary FA and proportions of circulating FA (% of total FA) were LA $\beta = 0.28$, ALA $\beta = 0.13$, EPA $\beta = 0.34$, and DHA $\beta = 0.45$. Body mass index (BMI), waist circumference, and presence of CVD influenced associations for LA; gender influenced LA, EPA, and DHA; alcohol intake influenced LA and DHA; and glucose tolerance status influenced ALA (p values interaction < 0.05). Coefficients for circulating FA as concentrations were LA $\beta = 0.19$, ALA $\beta = 0.10$, EPA $\beta = 0.31$, and DHA $\beta = 0.41$. **Conclusions:** This study suggests that characteristics such as BMI, alcohol intake, and expressing circulating FA as proportions or concentrations, influence associations between dietary and circulating FA.

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Introduction

Methods used to monitor dietary intake in population-based research often rely on self-reported dietary assessment. However, the subjective nature of self-reported dietary intake may lead to measurement error, which can result in obtaining inaccurate estimates of dietary intake and obscured associations with disease [1, 2].

For assessing total fat intake, no valid biomarker is available. Instead, proportions of circulating fatty acids (FA) are often used as biomarkers of intake of specific dietary FA [3, 4]. In particular, FA that are not (or in small amounts) synthesized endogenously, such as linoleic acid (LA; C18:2 n-6), alpha-linoleic acid (ALA; C18:3 n-3), eicosapentaenoic acid (EPA; C20:5 n-3) and docosahexaenoic acid (DHA; C22:6 n-3) correlate reasonably well with their respective reported intakes [4]. However, the relationship between dietary and circulating FA also tends to vary. In their review, Hodson et al. [4] reported correlation coefficients ranging from 0.01 to 0.77 for LA and from 0.21 to 0.69 for EPA.

The variation in correlation coefficients between studies may be explained by measurement error, time frame of data collected on both circulating and dietary FA, and by the influence of FA metabolism [3, 4]. The strength of associations between dietary and circulating FA may also vary between participants with different demographic, lifestyle, and health characteristics. One study concluded that associations were relatively constant regardless of characteristics such as age, gender, body weight, smoking, alcohol drinking, and disease status [5]. However, other studies concluded that gender, country of birth, smoking and disease status modified the association between circulating and dietary intakes of EPA and DHA, but not LA [6, 7]. Circulating FA are typically expressed as relative proportions (% of total FA). At present, it is unresolved whether levels of circulating FA should be expressed as relative proportions (% of total FA) or as absolute concentrations ($\mu\text{g/mL}$) [8–10].

In a cross-sectional analysis of data from 2 cohort studies, we investigated the influence of demographic, lifestyle, and health characteristics on the relation between self-reported dietary intake and circulating levels of LA, ALA, EPA, and DHA. We also explored differences between expressing levels of circulating FA as relative proportions and as absolute concentrations.

Methods

Study Populations

The current cross-sectional analysis is based on pooled data of participants from 2 Dutch cohorts; the Cohort study on Diabetes and Atherosclerosis Maastricht (CODAM study) and the Hoorn study. Details on the recruitment and selection of participants are given in online supplemental Data 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000486244). The study protocols were approved by the local medical Ethics Committees, and all participants gave their written informed consent before entering the study. The same research protocols were used in both studies for assessing dietary intake and circulating FA. From the 1,398 participants in the pooled cohorts, we excluded 198 participants with missing data on circulating FA (17 from CODAM; 181 from Hoorn), 20 participants with missing dietary intake data, 8 with extreme energy intake ($<2,092$ kJ/day [500 kcal] or $>14,644$ kJ/day [3,500 kcal] in women, $<3,347$ kJ/day [800 kcal] or $>16,736$ kJ/day [4,000 kcal] in men), and one with implausible values in the FA profile ($>100\%$). In total, 1,171 participants were included (469 from CODAM, 702 from Hoorn).

Dietary Intake Assessment

In both cohorts, dietary intake over the past year was assessed using a self-administered 79-item semi-quantitative food frequency questionnaire [11]. Dietary intakes were calculated using an extended version of the Dutch Food Composition table from 2001 (NEVO) [12]. For total fat intake, the relative validity of the questionnaire in a Dutch population was between 0.69 and 0.74 for men and between 0.58 and 0.63 for women when comparing the food frequency questionnaire with 12 monthly 24 h recalls [13].

Potential energy under-reporters were identified on the basis of disparities between reported energy intakes and predicted energy requirements, using Goldberg's cutoff point for the ratio of energy intake to basal metabolic rate ratio (EI:BMR <1.10), with physical activity level estimated to be 1.55 [14, 15].

Circulating FA Assessment

Venous blood samples were collected after overnight fast and stored at -80°C until use. FA composition in EDTA plasma (CODAM study) and serum (Hoorn study) was analyzed by gas liquid chromatography with flame ionization detection (AS Vitas, Oslo Innovation Center, Oslo, Norway) as described previously [16]. FA were expressed as relative proportions of the total area under the curve (% of total FA) and as absolute concentrations ($\mu\text{g/mL}$). In the present analyses, we studied the FA that are synthesized endogenously in limited amounts or not synthesized at all, that is, LA, ALA, EPA, and DHA.

Demographic, Lifestyle, and Health Characteristics

Self-administered questionnaires were completed on demographics, lifestyle, and medication use. CVD was defined as self-reported history of myocardial infarction, stroke, bypass surgery of the coronary arteries, balloon dilatation or stent placement, or the presence of signs of myocardial infarction or ischemia on an electrocardiogram, or the presence of an ankle-arm index <0.9 in either leg. All participants underwent a standard 75 g oral glucose tolerance test, except those with previously diagnosed diabetes. Participants were classified as having normal glucose tolerance (NGT), impaired glucose metabolism, or type 2 diabetes mellitus

(T2DM) according to the 1999 World Health Organization criteria [17]. Height (m) and body weight (kg) were measured with the participants wearing light clothing and no shoes. Waist circumference was measured with a tape midway between the lowest rib margin and the anterior superior spina iliaca.

Statistical Analysis

Participants' characteristics are presented as means $\pm$ SD, median (interquartile range), or percentages, depending on the type and distribution of variables. Prior to further analysis, self-reported dietary and circulating proportions of ALA, EPA, and DHA were log-transformed toward a normal distribution of the residuals.

As a proxy for correlation coefficients, we calculated standardized linear regression coefficients (95% CI) for the association of circulating FA (% of total FA) as the dependent variable, with self-reported dietary FA (% of total energy intake) as the independent variable, adjusted for original cohort. To assess the influence of demographic, lifestyle, and health characteristics on the standardized regression coefficients, dummy variables were created for the characteristics and interaction terms between dietary FA and the dummy variables were tested for significance in the regression models. To create dummy variables, the characteristics were categorized as follows: gender into men and women; age (y) and physical activity (min per week $\times$ intensity) into tertiles; smoking status into never, former, and current smokers; alcohol intake into no (<0.1 g/day), moderate (<30.0 g/day for men or <20.0 g/day for women), and high (≥ 30.0 g/day for men and ≥ 20.0 g/day for women); body mass index (BMI) into normal (<25 kg/m²), overweight (25–30 kg/m²), and obese (>30 kg/m²); waist into normal (<94 cm for men and <80 cm for women) increased (94–102 cm for men and 80–88 cm for women), and high (>102 cm for men and >88 cm for women); lipid-lowering medication and CVD into no and yes; glucose tolerance status into NGT, impaired glucose metabolism, or T2DM; and energy reporting as normal and under-reporters.

We subsequently explored the association of each participant characteristic on proportions of circulating LA, ALA, EPA, and DHA. The proportion of the total variation accounted for by each determinant was calculated by R² change in multivariable linear regression analysis. This model also controlled for cohort, total energy intake (kJ) and intakes from carbohydrate, protein, saturated, mono-unsaturated, and trans FA (%En). Two-sided *p* value <0.05 was considered to be statistically significant. To address the question on how to express and interpret levels of circulating FA, regression coefficients between circulating FA expressed as relative proportions and as absolute concentrations were calculated, and all analyses were repeated with circulating FA expressed as absolute concentrations (μ g/mL). All analyses were performed using IBM SPSS Statistics 21 for Windows 7.

Results

The mean age of the population was 68 ± 7 years, 54% were men, and mean BMI was 28.0 ± 4.2 kg/m². Of the participants, 32% never smoked, 35% had T2DM, 49% had prevalent CVD or a history of CVD, and 47% used anti-hypertensive drugs (Table 1).

Table 1. Baseline characteristics of the study population

	Total population (<i>n</i> = 1,171)
Age, years	67.9 $\pm$ 7.1
Gender, men, %	53.9
BMI, kg/m ²	28.0 $\pm$ 4.2
Waist circumference, %	
Normal	19.0
Increased	26.0
High	55.0
Physical activity, min/week*intensity	6.2 $\pm$ 4.7
Smoking status, %	
Never	31.5
Former	51.1
Current	16.7
Education level (low) [†] , %	50.5
Cardiovascular disease (yes), %	49.9
Glucose tolerance status, %	
Normal glucose tolerance	40.2
Impaired glucose metabolism	24.9
T2DM	34.9
Medication use (yes), %	
Anti-hypertensives	46.9
Lipid lowering	27.8
Dietary intake	
Energy intake, MJ/day	8.4 $\pm$ 2.2
EI:BMR	1.35 $\pm$ 0.34
Total protein (%En)	15.7 $\pm$ 2.4
Total carbohydrates (%En)	45.5 $\pm$ 6.7
Total fat (%En)	34.7 $\pm$ 5.5
SAFA (%En)	13.7 $\pm$ 2.7
MUFA (%En)	10.9 $\pm$ 2.2
PUFA (%En)	6.6 $\pm$ 1.8
TFA (%En)	1.1 $\pm$ 0.3
Alcohol intake	
No	15.9
Moderate	67.4
High	16.7

^a Values are presented as mean $\pm$ SD or *n* (%).

[†] Only assessed in Hoorn cohort.

BMI, body mass index; T2DM, type 2 diabetes mellitus; %En, percentage of energy; SAFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, poly-unsaturated fatty acids; TFA, trans fatty acids; LA, linoleic acid; ALA, alpha-linoleic acid; EPA eicosapentaenoic acid; DHA, docosahexaenoic acid; EI:BMR, ratio of EI (energy intake) to BMR (basal metabolic rate). Waist circumference was categorized as normal (<94 cm for men and <80.0 cm for women), increased (94–102 cm for men and 80–88 cm for women), and high (>102 cm for men and >88 cm for women). Alcohol intake was categorized as no (<0.1 g/day), moderate (<30.0 g/day for men or <20.0 g/day for women), and high (≥ 30.0 g/day for men and ≥ 20.0 g/day for women).

Table 2. Associations between circulating (% of total FA) and self-reported intakes (%En) of LA, ALA, EPA, and DHA, stratified for demographic, lifestyle, and health characteristics

	<i>n</i>	LA		ALA [†]		EPA		DHA	
		β (95% CI)	<i>p</i> value [‡]	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value	β (95% CI)	<i>p</i> value
Dietary FA (%En)		5.5 (1.7) ^b		0.43 (0.35 to 0.54)		0.02 (0.01–0.03)		0.05 (0.02–0.07)	
Circulating FA (% of total FA)		27.0 (3.7)		0.54 (0.46 to 0.65)		0.68 (0.50–0.94)		1.76 (1.40–2.18)	
Overall association	1,171	0.308 (0.253–0.363)		0.129 (0.072 to 0.186)		0.335 (0.281–0.390)		0.445 (0.394–0.496)	1.169
Gender [§]									
Men	631	0.358 (0.282–0.434)	Ref.	0.097 (0.023 to 0.171)	Ref.	0.400 (0.323–0.478)	Ref.	0.494 (0.424–0.564)	Ref.
Women	540	0.249 (0.170–0.328)	0.051	0.177 (0.093 to 0.261)	0.159	0.270 (0.195–0.344)	0.018	0.394 (0.320–0.469)	0.056
Age									
Tertile 1	384	0.277 (0.178–0.377)	Ref.	0.149 (0.044 to 0.253)	Ref.	0.392 (0.286–0.499)	Ref.	0.492 (0.395–0.589)	Ref.
Tertile 2	387	0.349 (0.239–0.460)	0.297	0.146 (0.028 to 0.264)	0.968	0.377 (0.250–0.504)	0.834	0.414 (0.309–0.519)	0.235
Tertile 3	400	0.293 (0.180–0.406)	0.822	0.100 (–0.011 to 0.211)	0.488	0.286 (0.186–0.386)	0.118	0.457 (0.355–0.559)	0.591
Smoking status									
Never	369	0.274 (0.174–0.375)	Ref.	0.096 (–0.004 to 0.197)	Ref.	0.320 (0.231–0.409)	Ref.	0.425 (0.344–0.506)	Ref.
Former	598	0.329 (0.238–0.420)	0.401	0.141 (0.054 to 0.228)	0.487	0.326 (0.237–0.415)	0.916	0.438 (0.353–0.522)	0.829
Current	195	0.315 (0.171–0.459)	0.613	0.169 (0.012 to 0.325)	0.398	0.473 (0.318–0.628)	0.071	0.534 (0.392–0.675)	0.164
Physical activity									
Tertile 1	387	0.342 (0.248–0.436)	Ref.	0.081 (–0.015 to 0.177)	Ref.	0.321 (0.237–0.406)	Ref.	0.449 (0.361–0.536)	Ref.
Tertile 2	386	0.273 (0.159–0.387)	0.316	0.157 (0.042 to 0.273)	0.278	0.302 (0.193–0.411)	0.776	0.482 (0.374–0.589)	0.614
Tertile 3	387	0.300 (0.189–0.410)	0.532	0.144 (0.033 to 0.254)	0.363	0.390 (0.275–0.505)	0.312	0.410 (0.309–0.511)	0.543
Alcohol intake									
No	186	0.153 (0.032–0.274)	Ref.	0.212 (0.078 to 0.347)	Ref.	0.278 (0.166–0.390)	Ref.	0.325 (0.219–0.431)	Ref.
Moderate	789	0.302 (0.221–0.384)	0.034	0.098 (0.010 to 0.186)	0.136	0.334 (0.254–0.414)	0.403	0.477 (0.403–0.551)	0.016
High	196	0.376 (0.194–0.557)	0.023	0.142 (–0.040 to 0.324)	0.468	0.347 (0.169–0.526)	0.465	0.428 (0.256–0.601)	0.258
BMI									
Normal	282	0.459 (0.352–0.565)	Ref.	0.169 (0.057 to 0.280)	Ref.	0.318 (0.208–0.427)	Ref.	0.415 (0.321–0.508)	Ref.
Overweight	588	0.259 (0.167–0.351)	0.003	0.136 (0.037 to 0.235)	0.639	0.384 (0.281–0.487)	0.339	0.501 (0.409–0.593)	0.163
Obesity	299	0.259 (0.124–0.395)	0.010	0.088 (–0.049 to 0.224)	0.305	0.272 (0.146–0.398)	0.542	0.381 (0.262–0.500)	0.627
Waist									
Normal	220	0.469 (0.345–0.593)	Ref.	0.189 (0.055 to 0.323)	Ref.	0.434 (0.298–0.570)	Ref.	0.461 (0.350–0.571)	Ref.
Increased	300	0.337 (0.191–0.484)	0.112	0.102 (–0.050 to 0.254)	0.320	0.302 (0.143–0.460)	0.137	0.435 (0.302–0.569)	0.742
High	636	0.221 (0.126–0.316)	0.001	0.121 (0.016 to 0.225)	0.382	0.313 (0.213–0.414)	0.126	0.429 (0.336–0.521)	0.633
Cardiovascular disease									
No	574	0.367 (0.289–0.444)	Ref.	0.182 (0.102 to 0.262)	Ref.	0.362 (0.279–0.445)	Ref.	0.467 (0.393–0.540)	Ref.
Yes	571	0.234 (0.157–0.312)	0.018	0.082 (0.005 to 0.160)	0.078	0.302 (0.228–0.376)	0.291	0.412 (0.340–0.485)	0.304
Lipid lowering medication									
No	846	0.363 (0.200–0.527)	Ref.	–0.024 (–0.191 to 0.143)	Ref.	0.305 (0.139–0.472)	Ref.	0.505 (0.350–0.660)	Ref.
Yes	325	0.321 (0.260–0.381)	0.503	0.095 (0.028 to 0.161)	0.057	0.329 (0.266–0.392)	0.711	0.457 (0.397–0.516)	0.411
Glucose tolerance status									
Normal glucose tolerance	467	0.333 (0.248–0.418)	Ref.	0.057 (–0.032 to 0.146)	Ref.	0.367 (0.274–0.459)	Ref.	0.457 (0.376–0.538)	Ref.
Impaired glucose metabolism	290	0.281 (0.160–0.401)	0.457	0.126 (–0.004 to 0.255)	0.355	0.318 (0.195–0.440)	0.496	0.444 (0.326–0.562)	0.845
T2DM	406	0.324 (0.224–0.424)	0.888	0.232 (0.130 to 0.334)	0.007	0.321 (0.223–0.420)	0.481	0.438 (0.345–0.532)	0.752

Table 2. (continued)

	n	LA		ALA [†]		EPA		DHA	
		β (95% CI)	p value [‡]	β (95% CI)	p value	β (95% CI)	p value	β (95% CI)	p value
Energy underreporting									
Normal reporters	906	0.300 (0.237–0.363)	Ref.	0.140 (0.076 to 0.205)	Ref.	0.355 (0.289–0.421)	Ref.	0.454 (0.393–0.514)	Ref.
Under-reporters	263	0.335 (0.222–0.447)	0.597	0.099 (–0.015 to 0.214)	0.541	0.291 (0.194–0.387)	0.282	0.430 (0.334–0.526)	0.684

^a Standardized β coefficients were derived from multivariable linear regression analyses with circulating FA (% of total FA), self-reported intake of FA (%En) and interaction terms (dietary FA intake*dummy variable), adjusted for original cohort.

^b Values are presented as means ± SD or medians (interquartile range).

[†] Circulating and dietary ALA, EPA, and DHA were log-transformed.

[‡] p values from interaction terms (dietary FA intake*dummy variable).

[§] Age was categorized as tertile 1 (48–65 years), tertile 2 (65–71 years), and tertile 3 (71–87 years); Physical activity was categorized as tertile 1 (<3.8 min/week*intensity), tertile 2 (3.8–7.0 min/week*intensity), and tertile 3 (>7.0 min/week*intensity); Alcohol intake was categorized as no (<0.1 g/day), moderate (<30.0 g/day for men or <20.0 g/day for women), and high (≥30.0 g/day for men and ≥20.0 g/day for women); BMI was categorized as normal (<25 kg/m²), overweight (25–30 kg/m²), and obese (>30 kg/m²); waist circumference was categorized as normal (<94 cm for men and <80.0 cm for women), increased (94–102 cm for men and 80–88 cm for women), and high (>102 cm for men and >88 cm for women). Energy under-reporters were defined as having EI:BMR <1.10 and normal energy reporters as having EI:BMR ≥1.10.

BMI, body mass index; T2DM, type 2 diabetes mellitus; LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; EI, energy intake; BMR, basal metabolic rate; %En, percentage of energy.

In the total population, standardized regression coefficients between dietary FA and relative proportions of circulating FA (% of total FA) were as follows: LA β = 0.31 (95% CI 0.25–0.36), ALA β = 0.13 (95% CI 0.07–0.19), EPA β = 0.34 (95% CI 0.28–0.39), and DHA β = 0.45 (95% CI 0.39–0.50; Table 2).

Several participant characteristics modified the strength of the association between dietary and circulating (% of total FA) LA. The association was stronger among participants with a normal BMI (β = 0.46; 95% CI 0.35–0.57) compared to overweight (β = 0.26; 95% CI 0.17–0.35, *p*_{int} = 0.003) or obese (β = 0.26; 95% CI 0.12–0.40, *p*_{int} = 0.01) participants, among participants with a normal (β = 0.47; 95% CI 0.34–0.59) vs. a high waist circumference (β = 0.22; 95% CI 0.13–0.32, *p*_{int} = 0.001), among participants without CVD (β = 0.37; 95% CI 0.29–0.44) vs. with CVD (β = 0.23; 95% CI 0.16–0.31, *p*_{int} = 0.02), and among participants with a higher (β = 0.38; 95% CI 0.19–0.56, *p*_{int} = 0.02) and moderate (β = 0.30; 95% CI 0.22–0.38, *p*_{int} = 0.03) alcohol intake vs no alcohol intake (β = 0.15; 95% CI 0.03–0.27).

For ALA, EPA, and DHA, fewer participant characteristics were identified that modified the strength of the association between dietary and circulating (% of total FA) FA. The association between dietary and circulating ALA was stronger among participants with T2DM (β = 0.23; 95% CI 0.13–0.33) than among participants with NGT (β = 0.06; 95% CI –0.03 to 0.25, *p*_{int} = 0.01). The association between dietary and circulating EPA was stronger among men (β = 0.40; 95% CI 0.32–0.48) than women (β = 0.27; 95% CI 0.19–0.35, *p*_{int} = 0.02), and the association between dietary and circulating DHA was stronger among participants with a moderate (β = 0.48; 95% CI 0.40–0.55, *p*_{int} = 0.02) vs. no alcohol intake (β = 0.33; 95% CI 0.22–0.43). Adjustment for additional demographic, lifestyle, health and dietary variables resulted in similar differences, except that the modifying effects of gender were more overt and statistically significant for ALA, EPA, and DHA (online supplement, Table 1).

To explore the nature of the differences in associations between participant characteristics, determinants of proportions of circulating LA, ALA, EPA, and DHA (% of total FA) were assessed by multivariable regression analyses. All demographic, lifestyle, health, and dietary variables together explained 25, 12, 18, and 27% of variation (total *R*²) in circulating LA, ALA, EPA, and DHA, respectively (Table 3). Self-reported intakes of LA, ALA, EPA, and DHA explained the largest part of the variations: 7, 2, 8, and 16% (partial *R*²), respectively.

Table 3. Demographic, lifestyle and health determinants of circulating LA, ALA, EPA, and DHA (% of total FA)^a

	LA			ALA [†]			EPA			DHA		
	β	<i>p</i> value	partial R ² , %	β	<i>p</i> value	partial R ² , %	β	<i>p</i> value	partial R ² , %	β	<i>p</i> value	partial R ² , %
Age, years [‡]	0.062	0.064	0.2	-0.044	0.223	0.1	0.109	0.002	0.7	0.183	<0.001	2.1
Gender (ref. = men)	-0.042	0.424	0.0	-0.156	0.006	0.6	0.054	0.323	0.1	0.009	0.856	0.0
Physical activity, min/week*intensity	0.046	0.100	0.2	0.041	0.182	0.1	-0.019	0.512	0.0	-0.010	0.713	0.0
Smoking status (ref. = never)												
Former	-0.020	0.531	0.0	-0.103	0.003	0.7	-0.019	0.584	0.1	-0.023	0.478	0.1
Current	-0.014	0.655		-0.068	0.044		0.010	0.753		0.014	0.660	
Alcohol intake (ref. = no)												
Moderate	0.003	0.943	0.1	-0.037	0.371	0.1	0.087	0.029	1.1	0.097	0.010	0.5
High	-0.052	0.356		-0.064	0.261		0.211	<0.001		0.132	0.010	
BMI, kg/m ²	-0.055	0.215	0.1	0.027	0.576	0.0	0.013	0.789	0.0	-0.039	0.372	0.1
Waist circumference (ref. = normal)												
Increased	-0.120	0.001	1.4	0.078	0.050	0.3	0.012	0.760	0.3	0.008	0.832	0.1
High	-0.216	<0.001		0.083	0.114		0.081	0.112		0.056	0.242	
Glucose tolerance status (ref. = normal)												
Impaired glucose metabolism	-0.082	0.006	1.2	0.020	0.540	0.1	0.009	0.768	1.6	0.020	0.496	0.2
T2DM	-0.126	<0.001		-0.013	0.715		-0.134	<0.001		-0.038	0.235	
Anti-hypertensive medication (ref. = no)	-0.005	0.874	0.0	-0.025	0.463	0.0	-0.040	0.215	0.1	-0.066	0.032	0.3
Lipid lowering medication (ref. = no)	-0.160	<0.001	1.9	-0.017	0.607	0.0	0.050	0.125	0.2	0.091	0.003	0.6
Cardiovascular disease (ref. = no)	-0.070	0.016	0.4	0.046	0.140	0.2	-0.007	0.828	0.0	-0.016	0.583	0.0
EI:BMR	-0.042	0.704	0.0	0.194	0.103	0.2	0.102	0.377	0.1	-0.013	0.904	0.0
Fatty acid intake (%En)												
LA	0.334	<0.001	6.8									
ALA [†]				0.161	<0.001	2.0						
EPA							0.313	<0.001	8.4			
DHA										0.433	<0.001	16.0
Model R ²			24.9			11.5			17.6			26.8

^a (Partial) R² derived from multivariable models for circulating FA (% of total FA) including original cohort, age, sex, BMI, waist circumference, physical activity score, smoking status, cardiovascular disease, glucose tolerance status, anti-hypertensive and lipid lowering medication, EI:BMR, alcohol intake, and the specific self-reported FA intake, additionally controlled for total energy intake and intakes from carbohydrate, protein, SFA, MUFA and TFA.

[†] Circulating and dietary ALA, EPA and DHA were log-transformed.

[‡] Alcohol intake was categorized as no (<0.1 g/day), moderate (<30.0 g/day for men or <20.0 g/day for women), and high (≥30.0 g/day for men and ≥20.0 g/day for women); waist circumference was categorized as normal (<94 cm for men and <80.0 cm for women), increased (94–102 cm for men and 80–88 cm for women), and high (>102 cm for men and >88 cm for women).

LA, linoleic acid; ALA, alpha-linoleic acid; EPA eicosapentaenoic acid; DHA, docosahexaenoic acid; EI, energy intake; %En, percentage of energy; BMR, basal metabolic rate.

Standardized regression coefficients between relative proportions (% of total FA) and absolute concentrations (μg/mL) of circulating FA were as follows: LA β = 0.39 (95% CI 0.34–0.44), ALA β = 0.84 (95% CI 0.81–0.86), EPA β = 0.93 (95% CI 0.90–0.95), and DHA β = 0.83 (95% CI 0.80–0.87). Standardized regression coefficients between dietary FA and absolute concentrations of circulating FA (μg/mL) were as follows: LA β = 0.19 (95% CI 0.13–0.24), ALA β = 0.10 (95% CI 0.04–0.16), EPA β = 0.31 (95% CI 0.25–0.36), and DHA β = 0.41 (95% CI 0.36–0.46). Expressing circulating FA as absolute concentrations (μg/mL) attenuated all modifying effects from participant characteristics on the associations with FA intake, except for the modifying effects of gender on EPA

and DHA, and alcohol on DHA (online suppl. Table 2). For circulating FA expressed as absolute concentrations (μg/mL), all variables together explained 13, 7, 19, and 25% of variation (total R²) in circulating LA, ALA, EPA, and DHA, respectively, and intake of LA, ALA, EPA, and DHA explained 2, 1, 6, and 12% (partial R²), respectively (online suppl. Table 3).

Discussion

Participant characteristics, such as BMI and alcohol intake, as well as the way of expressing circulating FA, as proportions or concentrations, influenced associa-

tions between self-reported dietary and circulating FA, in particular for LA. For each of the examined FA, its dietary intake was the primary, but not the only determinant of circulating FA. Other demographic, lifestyle, and health characteristics also influenced levels of circulating FA.

In agreement with earlier studies [4], the associations between dietary intakes and proportions of circulating LA, EPA and DHA were relatively strong ($\beta = 0.31$, $\beta = 0.34$, and $\beta = 0.45$, respectively) but weaker for ALA ($\beta = 0.13$). Although ALA is not synthesized endogenously, this weaker association may be explained by the rapid oxidation of dietary ALA in the body [18, 19] and by the lower precision of measuring ALA intake from the diet [20].

In general, associations between dietary and circulating FA may be attenuated by measurement errors and by FA metabolism [3, 4]. Earlier research shows that participants of older age, with higher BMI, lower level of education, and higher health consciousness, and women tend to under-report energy intake [21–23], which may affect the reliability of estimated self-reported FA intakes. On the other hand, factors such as smoking and age have been shown to affect proportions of circulating FA [24, 25], and some studies showed that women have more endogenous synthesis of EPA and DHA than men [26, 27]. These factors may cause weaker associations in some subgroups, or attenuate overall associations between reported dietary and circulating FA.

We observed that several participant characteristics substantially influenced the associations between reported dietary intakes and circulating proportions of FA. For example, in normal weight participants, the association between dietary and circulating proportions of LA was $\beta = 0.46$, which is generally considered ‘moderate’ for a biomarker of dietary intake, whereas the correlation was $\beta = 0.26$ in obese participants. These observed differences could be due to errors in the data on dietary or circulating FA, or due to biological differences in FA metabolism between non-obese and obese participants.

To explore the nature of the observed differences, we assessed the association of a range of participant characteristics with proportions of circulating FA. We did not find weaker associations in people who underreport total energy intake, although this has been suggested to be more prominent in obese people [28, 29]. Hence, energy underreporting is unlikely to fully explain the weaker associations in obese participants. For circulating LA, we observed that independent of self-reported LA intake, a high BMI, a high waist circumference, and an unfavor-

able health status were inversely associated with the proportion of LA in blood. Thus, participant characteristics may have an impact on FA metabolism and should be further explored. It is possible that delta 6-desaturase activity, the enzyme converting LA into gamma-linoleic acid, is altered in participants with insulin resistance, although at present there are no studies supporting this finding [30, 31]. Another explanation may be that in the presence of non-alcoholic fatty liver disease, hypertriglyceridemia, and insulin resistance, *de novo lipogenesis* is upregulated or FA oxidation is downregulated, promoting a higher proportion of SFA and MUFA in blood, and consequently a lower proportion of circulating LA [4, 32].

We also observed that older age was associated with higher proportions of circulating EPA and DHA, in line with earlier studies [24, 33]. Our observation that women had lower circulating proportions of ALA than men would be in agreement with studies showing that women have a larger capacity to convert ALA into EPA and DHA under the influence of sex hormones [26]. However, most women in our cohort were postmenopausal and the proportion of EPA and DHA did not differ between men and women. Thus, there must be other factors and unmeasured differences between men and women affecting the proportion of circulating poly-unsaturated FA. Overall, our data confirm earlier findings that participant characteristics and lifestyle factors other than diet may influence circulating proportions of FA [3, 4]. These factors should be taken into account when applying circulating FA as biomarkers of FA intake.

We also explored differences between expressing circulating FA as relative proportions or as absolute concentrations when using these as biomarkers of intake. We confirmed earlier findings that circulating FA as absolute concentrations ($\mu\text{g/mL}$) resulted in weaker associations with dietary intake than as proportions (% of total FA), in particular for LA [8, 9]. On the other hand, associations between self-reported LA and circulating LA expressed as absolute concentrations were less affected by alcohol intake, BMI, and waist circumference. This suggests that either our study had insufficient power to identify significant modifications when circulating LA was expressed as absolute concentration, or that associations between self-reported and circulating FA as absolute concentrations may be less dependent on participant characteristics. Thus, whether circulating FA can better be expressed as absolute concentrations or as relative proportions may depend on the research question.

Several limitations of our study should be mentioned. First, the observational nature of our study leaves room for reverse causation and residual confounding. For example, potential sources of residual confounding are socioeconomic status [34], which was not measured in one of our cohorts, and the background diet. The background diet may affect levels of specific circulating FA in 2 ways. It may be direct, for example, the uptake of omega-3 FA may be improved when consumed with a high fat meal [35]. It may also be indirect, for example, when other FA from the diet are elongated and desaturated and/or when FA are endogenously synthesized from carbohydrates and mixed into the circulating FA pool [36]. However, our additional analyses (online suppl. Table 1), including adjustments for background diet, did not result in different observations. The influence of these participant characteristics on the relation between dietary and circulating FA should be further studied in settings where dietary intakes are controlled. Second, we determined total FA in plasma (CODAM study) and serum (Hoorn study) instead of measuring separate blood lipid sub-fractions such as triglycerides, cholesteryl esters, phospholipids, or non-esterified FA. Proportions of FA differ between sub-fractions, and a change in the pool size of one sub-fraction, for example due to a disease, may potentially affect the total plasma or serum FA composition and thus complicate the interpretation of our results [4]. Also, each sub-fraction may reflect FA intake over different time frames before blood sampling and result in different associations with self-reported intakes. Thus, it might be of interest to also further study the influence of demographic, lifestyle, and health characteristics on the associations in different blood lipid sub-fractions and in adipose tissue. Third, information on intake of flaxseed or fish oil supplements was not collected. Food consumption data from the Netherlands show that in 2007–2010 0% of ALA and LA and 6% of total EPA and DHA intake originated from dietary supplements [37]. Consequently, in our study, the intake of EPA and DHA may have been slightly underestimated, which may have contributed to lower correlations between intake and blood markers.

In conclusion, our analysis suggests that demographic, lifestyle, health characteristics, and the way of expressing levels of circulating FA influence the strength of the associations between dietary and circulating FA. Better understanding of the factors that determine levels of circulating FA will help to improve their application as biomarkers of FA intake.

Ethics Statement

The study protocols of both cohorts were approved by the local medical Ethics Committees, and all participants gave their written informed consent before entering the study.

Disclosure Statement

A.J.W., M.A., and P.L.Z. are employed by Unilever R&D Vlaardingen, the Netherlands; S.E.M.D.H. was an MSc student at Unilever R&D and Wageningen University at the time of conducting the study. Unilever markets food products made of vegetable oils, including margarines and dressings. C.A.D. is a co-founder, stock-owner, board-member and consultant for the contract laboratory Vitas Ltd., in the Oslo Innovation Park, Oslo, Norway. Other authors declared no conflicts of interest to disclose.

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Author Contributions

The authors' contributions were as follows: C.J.K., C.G.S., C.D.A.S., J.M.D., and M.M.J.G. were the principal investigators of the Hoorn and CODAM study, and H.R., C.A.D., and A.E. enabled the fatty acid measurements. A.J.W., S.E.M.D.H., M.A., E.J.M.F., G.J.W., P.L.Z., J.M.D., and M.M.J.G. designed the present study and A.J.W., S.E.M.D.H., P.L.Z., J.M.D., and M.M.J.G. analyzed and interpreted the data, and A.J.W. wrote the manuscript. All authors reviewed the manuscript and provided comments. All authors approved of the final manuscript.

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