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Original Research

Measurement of Circulating Phospholipid Fatty Acids: Association between Relative Weight Percentage and Absolute Concentrations

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Key words: absolute concentration, correlation, partial correlation, phospholipid fatty acid, relative concentration, semipartial correlation

Objective: Most epidemiologic studies of circulating phospholipid fatty acids (PLFAs) and disease risk have used the relative concentration (percentage of total) of each fatty acid as the measure of exposure. Using relative concentrations, the total of all fatty acids is summed to 100% and thus the values of individual fatty acid are not independent. This has led to debate, along with the suggestion to use absolute concentrations of fatty acids. We aimed to examine the relationship between relative (weight percentage) and absolute (mg/L) concentrations of individual circulating PLFAs.

Methods: Relative and absolute concentrations of 41 circulating PLFAs were measured by gas chromatography in samples from 3 diverse populations. Correlations between the relative and absolute concentrations for each fatty acid were used to measure agreement. Unadjusted correlations and correlations adjusting absolute PLFA concentrations for total cholesterol were calculated.

Results: Unadjusted correlations between relative and absolute concentrations, as well as correlations adjusting absolute PLFA concentrations for total cholesterol, were high for most PLFAs in all 3 studies. Across the 3 studies, 28 of the 41 analyzed PLFAs had unadjusted correlations > 0.6 and 39 had adjusted correlations > 0.6.

Conclusions: Choice of relative vs absolute concentration may not affect interpretation of results for most circulating PLFAs in studies of association between individual PLFAs and disease outcomes, especially if a covariate reflecting total lipids, such as total circulating cholesterol, is included in the model. However, for fatty acids, such as 16:0 (palmitic acid), with low correlation between the 2 metrics, using relative vs absolute concentration may lead to different inferences regarding their association with the outcome. Because both concentrations could be obtained simultaneously from the same laboratory assay, use of both metrics is warranted to better understand PLFA–disease relationships.

INTRODUCTION

All cells and most bodily fluids contain fatty acids, whose composition is affected by both diet and metabolism. Epidemiologic studies have evaluated the association between fatty acids and risk of cancers, diabetes, and cardiovascular diseases [1–7]. Many have measured circulating phospholipid fatty acids (PLFAs) using gas chromatography (GC) and have expressed each fatty acid as either weight or molar percentage

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Abbreviations: AGES–Reykjavik = Age, Gene/Environment Susceptibility–Reykjavik, CARB = Carbohydrates and Related Biomarkers Study, CV = coefficient of variation, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, GC = gas chromatography, PLFA = phospholipid fatty acid, QC = quality control

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of total fatty acids analyzed. Using this approach, the total of all fatty acids must sum to 100% and thus the values of individual fatty acid are not independent. This has led to debate, along with the suggestion to use absolute concentrations as measures of fatty acids [8–12]. However, persons with high plasma lipoprotein concentrations will also have high absolute plasma PLFA concentrations because phospholipids in circulation are predominantly associated with lipoproteins as a component of the surface monolayer [13,14]. Therefore, the effect of any individual fatty acid measured in absolute concentration may be obscured by the total amount of lipids in plasma. Accepting that both approaches—relative and absolute—have limitations, it is unknown whether the choice of metric would affect study findings when circulating PLFAs are used as biomarkers of either exposure or outcome in epidemiologic studies.

Here we examine, in 3 independent data sets, the correlations between relative (weight percentage of total PLFAs analyzed) and absolute concentrations of circulating PLFAs. We hypothesized that these correlations may differ by fatty acid, and the results of our study could inform whether and for which fatty acids the choice of metric should be of concern.

MATERIALS AND METHODS

Study Samples

Samples were from 3 different studies: the Age, Gene/Environment Susceptibility–Reykjavik (AGES–Reykjavik) Study [15], the Carbohydrates and Related Biomarkers (CARB) Study [16], and the Uganda Omega-3 Trial. The AGES–Reykjavik was a population-based cohort study of men and women aged 66–96 at baseline (2002–2006); the present study included a subset of 279 baseline plasma samples analyzed for an ancillary study [17]. The CARB study was a randomized crossover feeding trial in healthy, nonsmoking men and women, aged 18–45. Serum samples from 60 participants collected at baseline (2006–2009) were available for the present analysis. The Uganda Omega-3 trial was a randomized, double-blind, placebo-controlled trial testing the effects of fish oil supplementation on men and women infected with both human immunodeficiency virus and human herpesvirus 8. Plasma samples from 66 participants collected at baseline (2012) were used for the present study. Fifty-five (83%) of the participants were also Kaposi's sarcoma positive. Serum/plasma samples were stored at −80°C until analysis and were on dry ice during shipment to testing labs. All studies were conducted under approval of their local institutional review boards and all participants provided written, informed consent. Analyses of blood performed for this study were also approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Fatty Acids Analysis

Total lipids were extracted from 200-μl plasma/serum using the method of Folch et al. [18]. An internal standard, 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine (21:0 PC, Avanti Polar Lipids, Inc., Alabaster, AL), was added to each sample before lipid extraction. The total lipid extract was evaporated under nitrogen, reconstituted in 80 μl of chloroform, and applied as a band onto an individual lane of one-dimensional thin-layer chromatography plates (TLC Silica gel 60 plates, EMD Millipore, Billerica, MA) using an Automatic TLC Sampler 4 (Camag Scientific, Wilmington, NC). After the plate was developed for half an hour in 45:10:0.5 hexane with 0.0005% BHT/ethyl ether/glacial acetic acid and allowed to dry briefly, the phospholipid fractions, which uniquely remained at the origin, were scraped off with a razor onto weighing paper and transferred to glass tubes for direct transesterification to prepare fatty acid methyl esters using the method of Lepage and Roy [19]. The fatty acid methyl ester samples were then injected in split mode (1:30) onto a GC system (5890 GC Series 2 with flame ionization detector, Agilent Technologies, Pal Alto, CA). The GC column used was a 100 m × 0.25 mm internal diameter fused silica capillary column with 0.2-μm coating (SP-2560 Supelco, Bellefonte, PA). The GC parameters were as follow: initial oven temperature 160°C for 19 minutes, ramp of 3.5°C/min to 245°C, and held for 15 minutes; injector temperature 240°C; detector temperature 275°C. The carrier gas was helium at 50 PSI for 15 minutes, ramp of 1 PSI/min to 75 PSI; make-up gas was nitrogen with a combined flow of 34 ml/min.

This GC method quantified 41 known fatty acids for this study. The relative concentration of each fatty acid was expressed as a weight percentage of total PLFAs analyzed. Absolute concentration (mg/L) of each fatty acid was calculated by comparing its peak area to that of the internal standard: mg/L $_{21:0}$ × (peak area count$_{\text{fatty acid of interest}}$/peak area count$_{21:0}$). A lab quality control (QC) sample (a pooled plasm) was included with each batch of study samples, and no obvious assay drift was observed. Concentrations of circulating PLFAs in the QC sample were similar to the average of the CARB samples. When measured in weight percentage, coefficients of variation (CVs) of the 41 PLFAs in the QC samples ranged from 0.4 to 15.6%; when measured in milligrams per liter, they ranged from 1.9 to 15.9%. All but one (18:1n8c) relatively abundant PLFAs (>0.1%) in the QC sample had CVs less than 6.1% whether measured in weight percentage or milligrams per liter; 18:1n8c had CVs of 9.5 and 10.2% measured in weight percentage and milligrams per liter, respectively.

Other Laboratory Assays

For the AGES–Reykjavik Study, total serum cholesterol was analyzed on a Hitachi 912, using reagents from Roche Diagnostics (Mannheim, Germany) following the

manufacturer's instructions. For the CARB and Uganda studies, total cholesterol was measured on a Roche Cobas Mira Plus Chemistry Analyzer, using reagents from Sekisui Diagnostics (Lexington, MA) following the manufacturer's instructions.

Statistical Analyses

Means were calculated for each fatty acid, measured as relative and absolute concentrations, and variances were given as the sample CV to allow comparison of variability across different fatty acids using a uniform metric. Pearson correlations were used to measure agreement between relative and absolute concentrations of each fatty acid; Spearman correlations were nearly identical to Pearson correlations and are therefore not given in the results. Scatter plots were used to visualize the relationship of Pearson correlations with relative concentrations and with sample CVs of relative concentrations. Relative concentrations were natural log-transformed in the scatter plots to aid visualization. Locally weighted scatter plot smoothing (LOWESS) smoothers are shown in the scatter plots. Because phospholipids in circulation are predominantly associated with lipoproteins as a component of the surface monolayer and total cholesterol is an important measure of lipoproteins reflecting total lipids, we calculated cholesterol-adjusted PLFA concentrations using the residual method with linear regression [20]. Semipartial correlations—the correlation between relative concentration and cholesterol-adjusted absolute concentration—were then calculated. Partial correlations—the correlation between relative and absolute concentrations both adjusted for total cholesterol—were also calculated. Simple linear regression was performed for each study using absolute concentration of total PLFAs as the dependent variable and cholesterol as the independent variable; R^2 from such linear regression models estimated the extent that variation in cholesterol concentration explained the variation in absolute concentration of total PLFAs analyzed. Statistical analyses were performed using SciPy Stack version 0.12.0 downloaded from SciPy.org or StataSE 13 (StataCorp LP, College Station, TX).

RESULTS

The 3 populations in the present analyses were from 3 different continents with distinct characteristics (Table 1). Participants in the AGES–Reykjavik Study were older, with higher total cholesterol concentrations, and had a higher proportion of females than in the other 2 studies.

Unadjusted correlations of the relative and absolute concentrations were high (>0.6) for most (28 of the 41 analyzed) circulating PLFAs in all 3 studies (Tables 2–4). An additional 11 PLFAs in the AGES–Reykjavik Study (Table 2) and 6 in the Uganda study (Table 4) had correlations over 0.6; 5 were the same PLFAs in the 2 studies. Variances, given as sample CV, of the absolute concentrations were higher than those of the relative concentrations in all 3 studies (Tables 2–4). Among the 41 PLFAs analyzed, fatty acids with higher variance (sample CV) tended to have higher correlations (Fig. 1, right panel). Above the threshold of about 0.1% (−2.3 on the natural log scale; Fig. 1, left panel) relative concentration, fatty acids with higher mean concentrations tended to have lower correlations.

Absolute concentration of total PLFAs analyzed was significantly associated with that of cholesterol (data not shown). R^2 from linear regression models indicated that variations in cholesterol explained 70, 46, and 60% of the variations in total PLFAs absolute concentrations in the AGES–Reykjavik, CARB, and Uganda studies, respectively. Cholesterol adjustment of absolute concentrations of individual PLFAs reduced their variances, making them similar or closer to the variances of the relative concentrations in all 3 studies (Tables 2–4). Semipartial correlations, the correlations between relative

Table 1. Characteristics of Participants from 3 Independent Studies[a]

	AGES–Reykjavik ($n = 279$)	CARB–Seattle ($n = 60$)	Uganda ($n = 66$)
Country of residence	Iceland	United States	Uganda
Age	77.9 (5.8)	29.4 (8.4)	39.4 (9.2)
BMI	26.7 (3.9)	27.7 (5.8)	23.3 (5.1)
Sex			
Male	105 (37.6%)	31 (51.7%)	37 (56.1%)
Female	174 (62.3%)	29 (48.3%)	28 (43.9%)
Total PLFA measured (mg/L)	1343.7 (207.9)	1417.2 (289.0)	1255.4 (272.9)
Total cholesterol (mg/dl)	215.7 (42.2)	164.9 (35.4)	158.4 (41.9)

BMI = body mass index, PLFA = phospholipid fatty acid.

[a]Data are means (SD) or frequency (%).

Table 2. Relative and Absolute Concentrations of Plasma Phospholipid Fatty Acids in the AGES–Reykjavik Study ($n = 279$).

	Relative wt% Concentration		Absolute Concentration					
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Cholesterol-Adjusted CV (%)	Pearson Coefficient[a]	Semipartial Correlation Coefficient[b]	Partial Correlation Coefficient[c]
Omega-3 fatty acids								
18:3n3	0.22	32.2	2.98	38.3	34.5	0.90	0.94	0.95
20:3n3	0.03	26.0	0.40	28.0	25.1	0.81	0.93	0.93
20:5n3	2.78	58.3	37.33	60.3	58.6	0.96	0.98	0.98
22:5n3	1.12	18.1	15.06	24.3	19.5	0.76	0.89	0.89
22:6n3	6.11	24.3	82.04	28.9	25.4	0.83	0.93	0.93
Omega-6 fatty acids								
18:2n6	17.75	15.8	238.75	22.8	18.2	0.72	0.87	0.87
18:3n6	0.05	59.0	0.62	61.6	61.3	0.96	0.98	0.98
20:2n6	0.33	14.6	4.44	23.2	18.7	0.73	0.87	0.87
20:3n6	2.26	32.2	30.51	37.0	35.2	0.90	0.96	0.96
20:4n6	6.80	22.3	90.74	23.4	22.6	0.77	0.88	0.92
22:2n6	0.06	22.7	0.78	26.2	21.0	0.80	0.91	0.92
22:4n6	0.21	26.8	2.78	29.2	27.6	0.85	0.94	0.94
22:5n6	0.07	34.7	1.00	38.8	37.1	0.91	0.96	0.96
Monounsaturated fatty acids								
16:1n9c	0.07	21.8	0.97	28.5	26.4	0.83	0.94	0.94
16:1n7c	0.55	32.1	7.53	39.4	36.8	0.92	0.96	0.97
17:1n9c	0.26	20.9	3.54	26.4	23.3	0.79	0.91	0.91
18:1n8c	0.20	26.2	2.74	30.9	26.7	0.85	0.93	0.94
18:1n9c	8.62	16.2	116.34	24.2	20.3	0.77	0.91	0.91
18:1n7c	1.27	15.2	16.98	19.9	17.5	0.63	0.84	0.87
18:1n5c	0.14	46.1	1.96	50.4	47.9	0.95	0.98	0.98
20:1n9	0.17	44.4	2.24	44.8	43.8	0.93	0.97	0.97
24:1n9	2.89	19.0	38.66	23.1	18.3	0.73	0.89	0.89
Saturated fatty acids								
14:0	0.39	22.2	5.25	29.3	22.6	0.85	0.89	0.92
15:0	0.20	20.2	2.64	27.0	20.1	0.82	0.87	0.90
16:0	27.17	4.3	364.91	15.8	9.4	0.20	0.42	0.43
17:0	0.41	16.9	5.54	23.4	17.9	0.73	0.86	0.87
18:0	13.32	7.0	179.23	17.6	11.3	0.48	0.66	0.66
20:0	0.60	14.8	8.03	21.7	13.5	0.69	0.78	0.81
22:0	1.64	14.3	22.04	21.6	11.6	0.70	0.72	0.77
23:0	0.75	15.3	10.15	22.3	12.9	0.73	0.75	0.81
24:0	1.29	16.1	17.35	22.8	14.4	0.73	0.80	0.84
Trans-fatty acids								
16:1n9t	0.04	37.6	0.60	40.3	39.1	0.92	0.97	0.97
16:1n7t	0.29	23.5	3.94	30.4	23.8	0.86	0.89	0.92
18:1n10-12t	0.08	45.1	1.12	48.9	47.0	0.94	0.98	0.98
18:1n9t	0.17	48.0	2.35	51.7	50.2	0.95	0.98	0.98
18:1n8t	0.09	45.5	1.20	48.3	47.3	0.93	0.97	0.97
18:1n7t	0.27	27.7	3.68	32.6	28.6	0.87	0.94	0.95
18:1n6t	0.20	34.8	2.73	38.1	35.6	0.91	0.96	0.96
18:2n6tt	0.04	23.6	0.54	27.1	24.4	0.82	0.93	0.93
18:2n6ct	0.06	30.9	0.79	37.4	33.0	0.91	0.94	0.96
18:2n6tc	0.06	31.4	0.82	38.5	34.1	0.91	0.95	0.96

AGES = Age, Gene/Environment Susceptibility, CV = coefficient of variation.

[a]Correlation of weight percentage relative concentration and absolute concentration.

[b]Correlation of weight percentage relative concentration and the cholesterol adjusted absolute concentration.

[c]Correlation with both relative and absolute fatty acid concentrations adjusted for cholesterol concentration.

concentrations and cholesterol-adjusted absolute concentrations, were higher than unadjusted correlations. Thirty-nine of the 41 PLFAs analyzed had semipartial correlations over 0.6 in all 3 studies. Only one circulating PLFA, 16:0, had a semipartial correlation less than 0.6 in all 3 studies: 0.42, 0.35, and 0.17 in the AGES–Reykjavik, CARB, and Uganda studies, respectively. Additional adjustment of relative PLFA concentrations for total cholesterol did not further improve correlations (i.e., partial correlation) between relative and absolute PLFA concentrations.

Table 3. Relative and Absolute Concentrations of Serum Phospholipid Fatty Acids in the CARB–Seattle Study Baseline Samples ($n = 60$)

	Relative wt% Concentration		Absolute Concentration					
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Cholesterol-Adjusted CV (%)	Pearson Coefficient[a]	Semipartial Correlation Coefficient[b]	Partial Correlation Coefficient[c]
Omega-3 fatty acids								
18:3n3	0.20	43.9	2.88	51.0	47.9	0.86	0.91	0.91
20:3n3	0.02	25.3	0.33	30.4	28.5	0.75	0.86	0.86
20:5n3	0.67	71.7	9.56	74.9	74.1	0.94	0.95	0.96
22:5n3	0.81	17.4	11.54	26.7	24.1	0.64	0.76	0.76
22:6n3	2.78	26.1	39.28	32.4	29.7	0.77	0.84	0.84
Omega-6 fatty acids								
18:2n6	22.32	12.3	315.05	22.2	18.5	0.46	0.64	0.64
18:3n6	0.08	80.5	1.25	102.4	101.4	0.97	0.98	0.98
20:2n6	0.39	12.9	5.53	24.3	19.7	0.54	0.65	0.65
20:3n6	2.83	27.8	40.59	39.2	36.8	0.87	0.90	0.90
20:4n6	10.69	16.8	151.02	25.6	21.1	0.59	0.68	0.68
22:2n6	0.04	24.5	0.52	27.6	23.8	0.72	0.81	0.81
22:4n6	0.43	20.6	6.16	31.8	28.5	0.75	0.83	0.83
22:5n6	0.28	28.7	4.05	37.9	35.8	0.84	0.89	0.89
Monounsaturated fatty acids								
16:1n9c	0.10	18.4	1.48	28.9	25.5	0.69	0.78	0.78
16:1n7c	0.41	42.9	6.03	65.9	60.1	0.94	0.93	0.95
17:1n9c	0.34	19.0	4.79	24.5	20.6	0.57	0.73	0.73
18:1n8c	0.20	41.2	2.78	51.0	49.9	0.91	0.94	0.95
18:1n9c	7.35	17.7	105.75	35.8	30.8	0.83	0.86	0.87
18:1n7c	1.14	13.9	16.20	26.8	21.9	0.57	0.69	0.69
18:1n5c	0.22	61.7	3.09	71.3	69.4	0.94	0.97	0.97
20:1n9	0.13	20.1	1.78	22.0	20.6	0.54	0.69	0.72
24:1n9	2.10	18.0	29.44	22.4	19.2	0.52	0.68	0.68
Saturated fatty acids								
14:0	0.30	24.5	4.23	34.5	30.8	0.79	0.84	0.85
15:0	0.16	22.2	2.30	26.3	26.0	0.70	0.75	0.79
16:0	25.25	4.4	358.08	21.3	16.1	0.29	0.35	0.35
17:0	0.38	14.1	5.30	19.8	19.0	0.45	0.58	0.63
18:0	13.86	8.0	197.19	23.8	19.1	0.55	0.62	0.62
20:0	0.50	16.4	7.03	21.5	18.3	0.48	0.63	0.64
22:0	1.61	17.2	22.47	21.4	16.8	0.52	0.65	0.65
23:0	0.72	17.8	10.06	25.2	19.9	0.63	0.69	0.70
24:0	1.43	17.8	19.95	22.0	16.2	0.52	0.65	0.65
Trans fatty acids								
16:1n9t	0.05	30.2	0.71	33.9	33.2	0.81	0.87	0.89
16:1n7t	0.17	23.3	2.38	28.1	27.5	0.75	0.81	0.83
18:1n10-12t	0.08	46.1	1.14	58.3	55.8	0.93	0.96	0.96
18:1n9t	0.13	43.3	1.85	54.9	52.0	0.92	0.95	0.95
18:1n8t	0.12	59.2	1.76	65.4	64.8	0.94	0.96	0.97
18:1n7t	0.25	37.8	3.56	45.6	44.3	0.88	0.93	0.93
18:1n6t	0.23	39.9	3.34	49.8	47.9	0.91	0.94	0.94
18:2n6tt	0.04	23.1	0.58	25.8	24.1	0.62	0.76	0.79
18:2n6ct	0.04	29.6	0.63	40.8	36.9	0.84	0.90	0.90
18:2n6tc	0.07	42.1	1.00	53.8	49.4	0.88	0.93	0.93

CARB = Carbohydrates and Related Biomarkers Study, CV = coefficient of variation.

[a]Correlation of weight percentage relative concentration and absolute concentration.

[b]Correlation of weight percentage relative concentration and the cholesterol adjusted absolute concentration.

[c]Correlation with both relative and absolute fatty acid concentrations adjusted for cholesterol concentration.

DISCUSSION

This methodologic study shows that relative (weight percentage) and absolute (mg/L) concentrations of most circulating PLFAs are highly correlated, especially after the absolute concentrations are adjusted for total circulating cholesterol concentration. For these highly correlated PLFAs, choice of metric may not affect study findings

Table 4. Relative and Absolute Concentrations of Plasma Phospholipid Fatty Acids in the Uganda Omega-3 Trial Baseline Samples ($n = 66$)

	Relative wt% Concentration		Absolute Concentration					
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Cholesterol-Adjusted CV (%)	Pearson Coefficient[a]	Semipartial Correlation Coefficient[b]	Partial Correlation Coefficient[c]
Omega-3 fatty acids								
18:3n3	0.15	41.3	1.96	55.6	48.9	0.89	0.92	0.94
20:3n3	0.02	46.1	0.25	55.4	46.1	0.85	0.91	0.93
20:5n3	0.80	51.0	10.08	59.5	57.0	0.91	0.96	0.96
22:5n3	1.06	22.4	13.29	31.3	25.7	0.72	0.85	0.85
22:6n3	4.13	28.9	51.48	33.2	27.6	0.79	0.89	0.89
Omega-6 fatty acids								
18:2n6	15.05	16.2	188.11	25.5	19.1	0.50	0.70	0.70
18:3n6	0.11	69.0	1.45	78.7	76.7	0.96	0.99	0.99
20:2n6	0.38	17.0	4.75	27.1	22.8	0.62	0.82	0.82
20:3n6	3.80	20.9	47.96	31.2	23.9	0.72	0.84	0.84
20:4n6	11.03	13.8	138.18	24.8	20.2	0.48	0.74	0.75
22:2n6	0.04	29.7	0.47	27.7	25.8	0.70	0.87	0.88
22:4n6	0.57	21.1	7.18	33.4	25.4	0.74	0.84	0.85
22:5n6	0.47	27.8	6.07	42.4	30.4	0.87	0.83	0.90
Monounsaturated fatty acids								
16:1n9c	0.12	23.1	1.45	32.2	28.7	0.73	0.88	0.90
16:1n7c	0.58	39.8	7.48	51.2	47.7	0.92	0.96	0.96
17:1n9c	0.24	23.9	2.96	29.6	26.4	0.66	0.81	0.82
18:1n8c	0.04	44.6	0.50	47.6	46.1	0.83	0.92	0.93
18:1n9c	10.15	17.0	129.18	33.5	27.6	0.78	0.90	0.90
18:1n7c	1.41	20.4	17.48	26.5	24.8	0.60	0.78	0.83
18:1n5c	0.02	29.5	0.30	29.8	28.1	0.66	0.80	0.82
20:1n9	0.14	18.3	1.71	25.1	22.5	0.53	0.75	0.80
24:1n9	2.02	23.8	24.79	23.7	18.6	0.58	0.82	0.82
Saturated fatty acids								
14:0	0.21	26.9	2.71	39.1	30.2	0.83	0.87	0.90
15:0	0.12	32.4	1.46	39.1	35.7	0.80	0.91	0.92
16:0	26.57	4.9	332.86	21.1	13.3	0.02	0.17	0.17
17:0	0.41	25.0	5.16	31.8	29.3	0.71	0.87	0.88
18:0	14.88	9.7	187.90	26.1	18.3	0.60	0.74	0.74
20:0	0.48	19.2	6.04	27.5	19.4	0.63	0.73	0.75
22:0	1.49	18.4	18.72	27.4	17.7	0.60	0.69	0.71
23:0	0.51	21.1	6.31	29.5	19.4	0.65	0.74	0.76
24:0	1.56	18.3	19.54	27.0	16.1	0.57	0.67	0.69
Trans-fatty acids								
16:1n9t	0.01	23.2	0.17	30.8	25.2	0.67	0.86	0.86
16:1n7t	0.14	58.2	1.77	52.0	51.3	0.88	0.93	0.94
18:1n10-12t	0.03	34.3	0.35	40.9	38.7	0.78	0.89	0.89
18:1n9t	0.04	33.6	0.47	40.5	40.6	0.81	0.90	0.92
18:1n8t	0.02	42.3	0.22	43.8	44.4	0.83	0.85	0.90
18:1n7t	0.13	72.7	1.62	73.9	71.8	0.90	0.94	0.94
18:1n6t	0.02	43.8	0.26	43.0	42.4	0.81	0.91	0.92
18:2n6tt	0.06	38.4	0.71	44.8	40.2	0.85	0.92	0.92
18:2n6ct	0.03	34.8	0.38	38.1	36.4	0.79	0.89	0.90
18:2n6tc	0.08	41.1	1.03	47.2	43.4	0.87	0.94	0.94

CV = coefficient of variation.

[a]Correlation of weight percentage relative concentration and absolute concentration.

[b]Correlation of weight percentage relative concentration and the cholesterol adjusted absolute concentration.

[c]Correlation with both relative and absolute fatty acid concentrations adjusted for cholesterol concentration.

when circulating PLFAs are used as biomarkers in epidemiologic studies. The present study was conducted in 3 diverse populations from 3 different continents that differed greatly in age, BMI, and health status. Although the PLFA profiles from the 3 data sets are representative of typical plasma PLFA profiles as reported by Hodson et al. [21], the diversity of the 3 populations was also reflected in the differences in specific PLFAs; for example, 20:5n3 (eicosapentaenoic acid, EPA) and 22:6n3 (docosahexaenoic acid, DHA) were substantially higher in

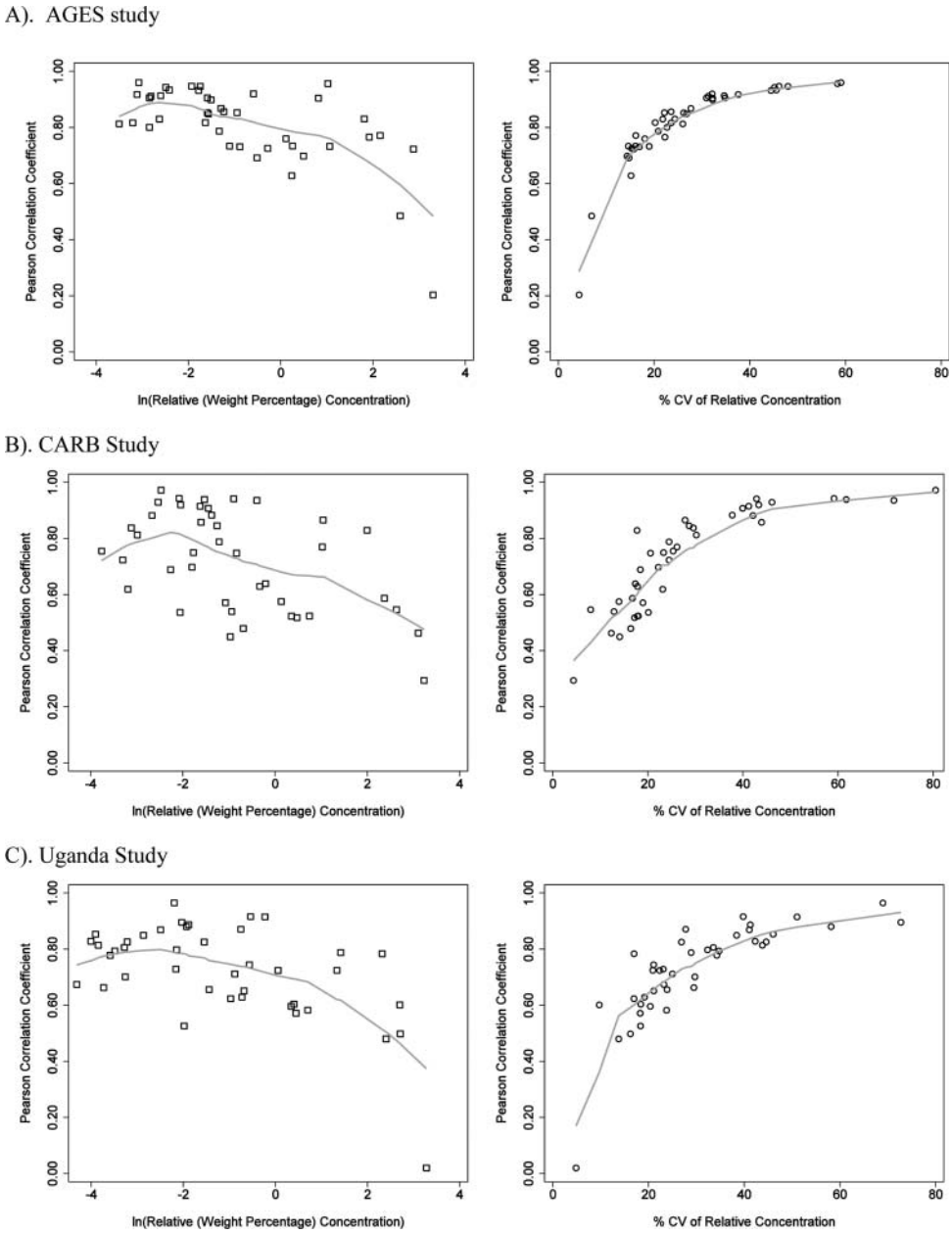

Figure 1. Relationship of Pearson correlations of relative (weight percentage) and absolute concentrations of 41 circulating PLFAs with mean relative concentrations (left panel, open squares) and with sample CVs of relative concentrations (right panel, open circles). The relative concentrations were natural log transformed on the *x*-axis of the scatter plots (left panel). LOWESS smoothers are shown in the scatter plots. (A) AGES study ($n = 279$); (B) CARB study ($n = 60$); (C) Uganda study ($n = 66$).

both relative and absolute concentrations in the AGES–Reykjavik participants, a population in which fish consumption and fish oil supplementation are common. In addition, the overall unadjusted correlations were higher in the AGES–Reykjavik Study than in the CARB and Uganda studies. To test whether the higher correlation seen in the AGES–Reykjavik Study was due to larger numbers of subjects, we randomly selected 2 subsets of 60 participants from the AGES–Reykjavik Study and the higher correlations remained.

Some differences in correlations of the 2 concentration metrics were observed across fatty acids and across studies. Correlations were reasonably consistent across studies for omega-3 fatty acids. The correlations of EPA, DHA, and α-linolenic acid (18:3n3), which are the most commonly investigated omega-3 fatty acids in epidemiologic studies, were high, especially after controlling for circulating cholesterol concentration (semipartial correlations 0.84–0.98). Correlations of omega-6 fatty acids were lower in the CARB and Uganda studies than in the AGES–Reykjavik Study. The correlations of linoleic

(18:2n6) and arachidonic (20:4n6) acids, which are the most commonly investigated omega-6 fatty acids in epidemiologic studies, were less than 0.6 in the CARB and Uganda studies but improved to 0.64–0.74 after controlling for cholesterol in these 2 studies. The semipartial correlation coefficients of linoleic and arachidonic acids in the AGES–Reykjavik Study were 0.87 and 0.88, respectively. Correlations of monounsaturated fatty acids had more variability across studies. However, the most commonly investigated monounsaturated fatty acids, palmitoleic (16:1n7c) and oleic (18:1n9c), had consistently high correlations in all 3 studies (semipartial correlations 0.86–0.96). Correlations of saturated fatty acids were in general lower than those of other classes of fatty acids. The most commonly investigated saturated fatty acid, palmitic (16:0), had consistently weak correlations in all 3 studies even after controlling for cholesterol (semipartial correlations 0.17–0.42). Trans-fatty acids had very low concentrations and mostly high correlations in all 3 studies.

One factor that influences the magnitude of correlation is the variability in the data. A more restricted range in values—that is, less variability reflected by smaller sample CVs—leads to lower correlation coefficients. The variances, given as sample CVs, of the absolute concentrations of individual PLFAs were higher than those of the relative concentrations. This could be explained in part by the fact that the range of the absolute concentrations was wide—the ratio of the highest to the lowest absolute concentration of total PLFAs was over 2-fold in each of the 3 data sets, and the sum of the relative concentrations is constrained to 100%. The concentrations of highly abundant fatty acids tended to have smaller sample CVs, especially when the concentrations were measured in weight percentage relative term. Palmitic acid (16:0) was the most abundant PLFA in all 3 studies and had the smallest sample CV. The unadjusted correlation coefficients of its relative and absolute concentrations were weak at 0.20, 0.29, and 0.02 in the AGES–Reykjavik, CARB, and Uganda Omega-3 studies, respectively; after adjusting the absolute concentrations for cholesterol, the semipartial correlations were still moderate at 0.42, 0.35, and 0.17, respectively.

The low correlation between the relative and absolute concentrations of 16:0 suggests that conclusions regarding its association with a disease outcome may differ depending on the metric used. 16:0 is the initial major product of the *de novo* lipogenesis pathway [22,23] and is in many food sources such as beef and hard cheese [24]. Its circulating levels are also affected by alcohol consumption [25]. Though the incorporation of dietary 16:0 into plasma phospholipids has been demonstrated using stable isotope tracers [26], the contribution of *de novo* synthesized 16:0 to circulating phospholipids 16:0 abundance is unknown. Studies have found that relative concentrations of plasma phospholipid 16:0 are positively associated with type 2 diabetes [27] and atrial fibrillation [28] but not associated with clinical coronary heart disease outcomes [22]. It would be interesting to evaluate the association between absolute concentrations of 16:0 and these disease outcomes.

Few studies have compared disease associations with individual circulating PLFAs measured as relative concentration vs measured as absolute concentration. Schwertner and Mosser showed that the mean concentrations of serum phospholipids 16:0 and 18:0 were significantly higher and that of 20:5n3 was significantly lower in individuals with coronary artery disease than in controls whether the fatty acids were measured in absolute or weight percentage relative concentrations [29]. However, a number of other fatty acids showed differential association with coronary artery disease status when different concentration metrics were used. The pattern observed was inconsistent with what our results would have predicted, which could be due to the facts that the study sample size was small (18 patients with coronary artery disease and 12 controls) and no covariate adjustment was done. In addition, the total serum PLFAs absolute concentration in their study was much higher than those in ours. The absolute concentrations of total PLFAs in our study were similar to those reported by Lindberg et al. [30,31] and were also consistent with the phospholipid concentrations of subjects from different geographic locations reported by Dougherty et al. [32].

Our study has several limitations. First, the 3 sets of samples were assayed separately instead of randomized together. However, a lab QC sample was included with each batch of study samples—no obvious assay drift was observed, and correlations between relative and absolute concentrations were calculated within each study population. Second, cholesterol was used as a surrogate for total circulating lipids. We chose to adjust for total cholesterol because it is an independent risk factor in many diseases and therefore is often already included in study models. Finally, we did not have a disease outcome in this study. To determine unequivocally whether metric matters, the association of PLFAs characterized as relative and absolute needs to be evaluated with specific disease outcomes. Our study of correlations between relative and absolute concentrations of PLFAs in 3 diverse populations provided a first step in our understanding of the relationship between the 2 metrics.

CONCLUSIONS

Results from 3 independent and diverse data sets indicate that relative (weight percentage) and absolute concentrations of most circulating PLFAs are highly correlated, especially after the absolute concentration is adjusted by circulating cholesterol concentration, which essentially controls for total lipids. These results suggest that, except for fatty acids such as 16:0 that have low correlation between the 2 metrics, choice of

relative vs absolute concentration may not matter for most circulating PLFAs in epidemiologic studies on the association between individual circulating PLFAs and disease outcomes, especially if appropriate covariates such as total circulating cholesterol are included in the models.

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REFERENCES

1. Shen XJ, Zhou JD, Dong JY, Ding WQ, Wu JC: Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. Br J Nutr 108:1550–1556, 2012.
2. Brasky TM, Darke AK, Song X, Tangen CM, Goodman PJ, Thompson IM, Meyskens FL Jr, Goodman GE, Minasian LM, Parnes HL, Klein EA, Kristal AR: Plasma phospholipid fatty acids and prostate cancer risk in the select trial. J Natl Cancer Inst 105:1132–1141, 2013.
3. Wu JH, Micha R, Imamura F, Pan A, Biggs ML, Ajaz O, Djousse L, Hu FB, Mozaffarian D: Omega-3 fatty acids and incident type 2 diabetes: a systematic review and meta-analysis. Br J Nutr 107 (Suppl 2):S214–S227, 2012.
4. Pan A, Chen M, Chowdhury R, Wu JH, Sun Q, Campos H, Mozaffarian D, Hu FB: Alpha-linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr 96:1262–1273, 2012.
5. Salter AM: Dietary fatty acids and cardiovascular disease. Animal 7(Suppl 1):163–171, 2013.
6. Schwingshackl L, Hoffmann G: Monounsaturated fatty acids and risk of cardiovascular disease: synopsis of the evidence available from systematic reviews and meta-analyses. Nutrients 4:1989–2007, 2012.
7. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, Franco OH, Butterworth AS, Forouhi NG, Thompson SG, Khaw KT, Mozaffarian D, Danesh J, Di Angelantonio E: Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. Ann Intern Med 160:398–406, 2014.
8. Chow CK: Fatty acid composition of plasma phospholipids and risk of prostate cancer. Am J Clin Nutr 89:1946; author reply 1946–1947, 2009.
9. Crowe FL: Reply to CK Chow. Am J Clin Nutr 89:1946–1947, 2009.
10. McCulloch M, Alvarez E, Gendreau R, Haney S, Harrelson J, Luo R: Re: Serum phospholipid fatty acids and prostate cancer risk in the select trial. J Natl Cancer Inst 106:dju024, 2014.
11. Brasky TM, Thompson IM, King IB, Kristal AR: Response. J Natl Cancer Inst 106:dju021, 2014.
12. Schwertner HA, Mosser EL: Lipid fatty acids calculated on a concentration vs percentage basis. Am J Clin Nutr 59:1093, 1994.
13. Kontush A, Chapman MJ: Lipidomics as a tool for the study of lipoprotein metabolism. Curr Atheroscler Rep 12:194–201, 2010.
14. Wiesner P, Leidl K, Boettcher A, Schmitz G, Liebisch G: Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. J Lipid Res 50:574–585, 2009.
15. Harris TB, Launer LJ, Eiriksdottir G, Kjartansson O, Jonsson PV, Sigurdsson G, Thorgeirsson G, Aspelund T, Garcia ME, Cotch MF, Hoffman HJ, Gudnason V: Age, Gene/Environment Susceptibility–Reykjavik Study: multidisciplinary applied phenomics. Am J Epidemiol 165:1076–1087, 2007.
16. Song X, Navarro SL, Diep P, Thomas WK, Razmpoosh EC, Schwarz Y, Wang CY, Kratz M, Neuhouser ML, Lampe JW: Comparison and validation of 2 analytical methods for measurement of urinary sucrose and fructose excretion. Nutr Res 33:696–703, 2013.
17. Reinders I, Song X, Visser M, Eiriksdottir G, Gudnason V, Sigurdsson S, Aspelund T, Siggeirsdottir K, Brouwer IA, Harris TB, Murphy RA: Plasma phospholipid PUFAs are associated with greater muscle and knee extension strength but not with changes in muscle parameters in older adults. J Nutr 145:105–112, 2015.
18. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226:497–509, 1957.
19. Lepage G, Roy CC: Direct transesterification of all classes of lipids in a one-step reaction. J Lipid Res 27:114–120, 1986.
20. Willett W: "Nutritional epidemiology," 3rd ed. New York: Oxford University Press, 2012.
21. Hodson L, Skeaff CM, Fielding BA: Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res 47:348–380, 2008.
22. Wu JH, Lemaitre RN, Imamura F, King IB, Song X, Spiegelman D, Siscovick DS, Mozaffarian D: Fatty acids in the de novo lipogenesis pathway and risk of coronary heart disease: the Cardiovascular Health Study. Am J Clin Nutr 94:431–438, 2011.
23. Chong MF, Fielding BA, Frayn KN: Metabolic interaction of dietary sugars and plasma lipids with a focus on mechanisms and *de novo* lipogenesis. Proc Nutr Soc 66:52–59, 2007.
24. Hu FB, Stampfer MJ, Manson JE, Ascherio A, Colditz GA, Speizer FE, Hennekens CH, Willett WC: Dietary saturated fats and

their food sources in relation to the risk of coronary heart disease in women. Am J Clin Nutr 70:1001–1008, 1999.

25. Siler SQ, Neese RA, Hellerstein MK: *De novo* lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption. Am J Clin Nutr 70:928–936, 1999.
26. Hodson L, McQuaid SE, Karpe F, Frayn KN, Fielding BA: Differences in partitioning of meal fatty acids into blood lipid fractions: a comparison of linoleate, oleate, and palmitate. Am J Physiol Endocrinol Metab 296:E64–E71, 2009.
27. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw KT, Wareham NJ, Forouhi NG: Fatty acids measured in plasma and erythrocyte–membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: A pilot study in the European Prospective Investigation into cancer And Nutrition (EPIC)–Norfolk Cohort. Am J Clin Nutr 92:1214–1222, 2010.
28. Fretts AM, Mozaffarian D, Siscovick DS, Djousse L, Heckbert SR, King IB, McKnight B, Sitlani C, Sacks FM, Song X, Sotoodehnia N, Spiegelman D, Wallace ER, Lemaitre RN: Plasma phospholipid saturated fatty acids and incident atrial fibrillation: the Cardiovascular Health Study. J Am Heart Assoc 3:e000889, 2014.
29. Schwertner HA, Mosser EL: Comparison of lipid fatty acids on a concentration basis vs weight percentage basis in patients with and without coronary artery disease or diabetes. Clin Chem 39:659–663, 1993.
30. Lindberg M, Saltvedt I, Sletvold O, Bjerve KS: Long-chain n-3 fatty acids and mortality in elderly patients. Am J Clin Nutr 88:722–729, 2008.
31. Lindberg M, Midthjell K, Bjerve KS: Long-term tracking of plasma phospholipid fatty acid concentrations and their correlation with the dietary intake of marine foods in newly diagnosed diabetic patients: results from a follow-up of the Hunt Study, Norway. Br J Nutr 109:1123–1134, 2013.
32. Dougherty RM, Galli C, Ferro-Luzzi A, Iacono JM: Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: a study of normal subjects from Italy, Finland, and the USA. Am J Clin Nutr 45:443–455, 1987.

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