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

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Abstract

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 are 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 three 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 three 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 two metrics, using relative vs. absolute concentration may lead to different inferences regarding their association with the outcome. Since both concentrations could be obtained

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simultaneously from the same laboratory assay, use of both metrics is warranted to better understand PLFA-disease relationships.

Keywords

Absolute concentration; Correlation; Partial correlation; Phospholipid fatty acid; Relative concentration; Semipartial correlation

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 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 as 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 three independent datasets, 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 three 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 subset of 279 baseline plasma samples analyzed for an ancillary study [17]. The CARB study was a randomized cross-over 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 (HIV) and human herpesvirus 8 (HHV8). Plasma samples from 66 participants collected at baseline (2012) were used for the present study. 55 (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 manuscript were also approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Fatty Acids Analysis

Total lipids were extracted from 200μl plasma/serum using the method of Folch [18]. Internal standard, 1,2-dihenarachidoyl-sn-glycero-3-phosphocholine (21:0 PC, Avanti Polar Lipids, Inc., Alabaster, Alabama), was added to each sample before lipid extraction. The total lipid extract was evaporated under nitrogen, reconstituted in 80μl of chloroform, and applied as a band onto an individual lane of one dimensional thin layer chromatography plate (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 [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 100m x 0.25mm internal diameter fused silica capillary column with 0.2μm coating (SP-2560 Supelco, Belefonte, PA). The GC parameters were: 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 combined flow of 34ml/min.

This GC method quantified 41 known fatty acids for this study. 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 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 mg/L, 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 mg/L; 18:1n8c had CVs of 9.5% and 10.2% measured in weight percentage and mg/L, 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) and 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) and 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 coefficient of variation (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; Spearmen correlations were nearly identical to Pearson correlations and are therefore not given in 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. LOWESS smoothers were shown in the scatter plots. Since 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 variation in cholesterol concentration explains the variation in the absolute concentration of total PLFAs analyzed. Statistical analyses were performed using SciPy version 0.12.0 (http://www.scipy.org/) or StataSE 13 (College Station, TX).

RESULTS

The three populations in the present analyses were from three different continents with distinct characteristics (Table 1). Participants in the AGES-Reykjavik study were older with higher total cholesterol concentrations and had higher proportion of females than in the other two studies.

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

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, bringing them similar or closer to the variances of the relative concentrations in all three studies (Tables 2, 3, and 4). Semipartial correlations, the correlations between relative concentrations and cholesterol-adjusted absolute concentrations, were higher than unadjusted correlations. 39 of the 41 PLFAs analyzed had semipartial correlation over 0.6 in all three studies. Only one circulating PLFA, 16:0, had a semipartial correlation less than 0.6 in all three 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.

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 when circulating PLFAs are used as biomarkers in epidemiologic studies. The present study was conducted in three very diverse populations from three different continents that differed greatly in age, BMI, and health status. Although the PLFA profiles from the three datasets are representative of typical plasma PLFA profiles as reported by Hodson *et al* [21], the diversity of the three populations was also reflected in the differences of specific PLFAs; for example 20:5n3 (eicosapentaenoic acid; EPA) and 22:6n3 (docosahexaenoic acid; DHA) were substantially higher in 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 two subsets of 60 participants from the AGES-Reykjavik study and the higher correlations remained.

Some differences in correlations of the two concentration metrics were observed across fatty acids and across studies. Correlations were reasonably consistent across studies for omega-3 fatty acids. The correlation 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 correlation 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 CARB and Uganda studies and but improved to 0.64 – 0.74 after controlling for cholesterol in these two 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 correlation in all three 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 correlation in all three 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 three studies.

One factor that influences the magnitude of correlation is the variability in the data. A more restricted range in values, i.e. less variability reflected by smaller sample CV, 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 two fold in each of the three datasets; while 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 three 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 its 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 of 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]. While 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 concentration 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 *et al* showed that the mean concentrations of serum phospholipid 16:0 and 18:0 were significantly higher, and 20:5n3 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 coronary artery disease patients 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 three 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 surrogate for total circulating lipids. We chose to adjust for total cholesterol because it is an independent risk factor of 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 if 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 three diverse populations provided a first step in our understanding of the relationship between the two metrics.

CONCLUSIONS

Results from three independent and diverse datasets 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 two metrics, choice of relative vs. absolute concentration may not matter for most circulating PLFAs in epidemiologic studies of 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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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

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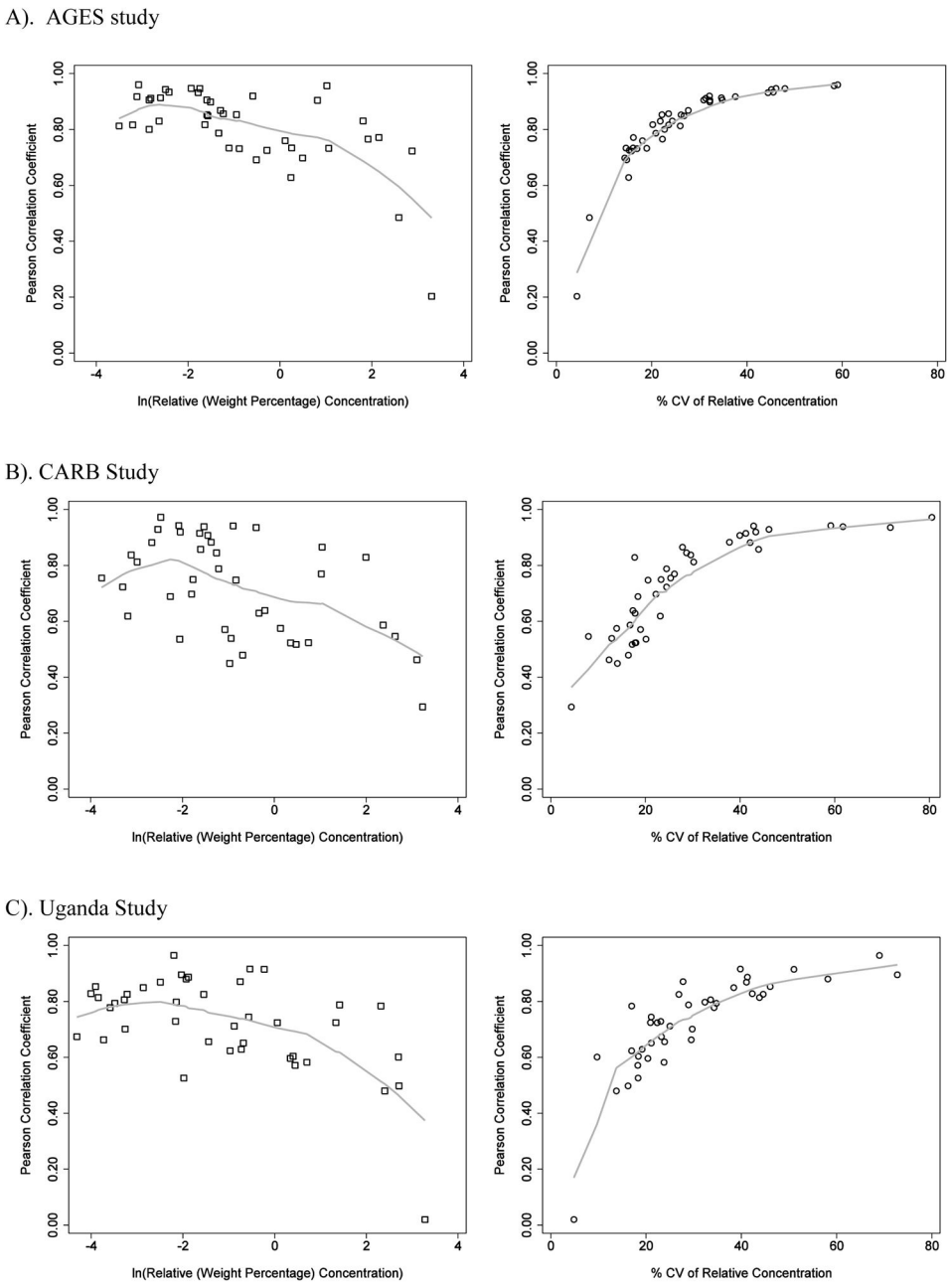

Figure 1.

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). PLFA, phospholipid fatty acid.

Table 1

Characteristics of Participants from Three Independent Studies [a]

	AGES-Reykjavik (n = 279)	CARB - Seattle (n = 60)	Uganda (n = 66)
County of residence	Iceland	U.S.A.	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)

[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 % conc.		Absolute conc.			Pearson Coef. [a]	Semipartial corr. coef. [b]	Partial corr. coef. [c]
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Chol adj CV (%)			
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

	Relative wt % conc.		Absolute conc.			Pearson Coef. [a]	Semipartial corr. coef. [b]	Partial corr. coef. [c]
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Chol adj CV (%)			
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

[a] Correlation of weight percent relative concentration and absolute concentration

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

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

Table 3

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

	Relative wt % conc.		Absolute conc.			Pearson Coef. [a]	Semipartial corr. coef. [b]	Partial corr. coef. [c]
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Chol adj CV (%)			
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

	Relative wt % conc.		Absolute conc.			Pearson Coef. [a]	Semipartial corr. coef. [b]	Partial corr. coef. [c]
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Chol adj CV (%)			
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

[a] Correlation of weight percent relative concentration and absolute concentration

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

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

Table 4

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

	Relative wgt % conc.		Absolute conc.			Pearson Coef. [a]	Semipartial corr. coef. [b]	Partial corr. coef. [c]
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Chol adj CV (%)			
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

	Relative wgt % conc.		Absolute conc.			Pearson Coef. [a]	Semipartial corr. coef. [b]	Partial corr. coef. [c]
	Mean (%)	Sample CV (%)	Mean (mg/L)	Sample CV (%)	Chol adj CV (%)			
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

[a] Correlation of weight percent relative concentration and absolute concentration

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

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

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