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Repeated versus single measurement of plasma omega-3 fatty acids and risk of heart failure

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Abstract

Purpose: Studies have previously examined the relation between a single measure of plasma fatty acids and risk of heart failure. However, it is unclear whether the use of repeated measures of fatty acids over time is required for the assessment of omega-3 fatty acids-heart failure relation.

Methods: Using a nested case-control design, this ancillary study used 421 cases and 421 matched controls from the Physicians' Health Study to assess the variability of plasma phospholipid fatty acids over time and compare the results of omega-3 fatty acids-heart failure associations using a single versus repeated measurements of plasma phospholipid fatty acids. Plasma omega-3 fatty acids were measured at baseline (1982) and approximately 15 years later using gas chromatography.

Results: Spearman correlation coefficients between baseline and follow-up measures of α -linolenic acid (ALA), EPA, DPA, and DHA were 0.20, 0.45, 0.28, and 0.50, respectively, in the control series. Multivariable adjusted odds ratios for heart failure per standard deviation higher plasma ALA were 0.98 (95% CI: 0.85-1.13) when using baseline ALA and 0.86 (95% CI: 0.74-1.01) when using the average of baseline and follow-up ALA measurements. Corresponding odds ratios for total long chain omega-3 FAs (EPA + DHA + DPA) were 0.87 (0.73-1.03) and 0.88 (0.75-1.04).

Conclusions: Our data demonstrate modest correlation between measurements of plasma phospholipid fatty acids spaced by 15 years. A single measurement of plasma phospholipid fatty acids appears reasonable to estimate the risk of heart failure over long-term follow up.

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Keywords

epidemiology; omega-3 fatty acids; methodology; heart failure

Introduction

Heart failure (HF) is still a major public health burden in the United States with high mortality [1-3]. Our group and others have previously reported positive associations of fatty acids (FAs) with incident HF or its risk factors [4-9]. In addition, the GISSI-HF trial found that an intervention with 1 gram of eicosapentaenoic acid (EPA) / docosahexaenoic acid (DHA) daily was associated with an 8% decrease in risk of death or hospital re-admittance for cardiovascular reasons in people with HF [7]. Due to costs and logistical issues associated with measuring blood FAs in a large number of subjects, a common limitation of previous observational studies has been the use of a single measurement of plasma or red blood cell membrane fatty acids. Plasma or red blood cell membrane fatty acid composition can change with dietary patterns and metabolism over time, thereby raising the question as to whether a single measure of plasma fatty acids is appropriate in epidemiologic studies. Non-differential exposure misclassification during follow-up may bias the results towards the null. Because it is expensive to measure fatty acid profiles, it is important to know if repeated measures of plasma fatty acids are necessary to make a valid inference on the relation of fatty acids with outcomes of interest.

A previous study assessing long-term reliability of plasma phospholipid measurement utilized two samples obtained approximately 3 years apart from 50 subjects and found correlation coefficients ranging from 0.35-0.51 for omega-3 FAs [10]. However, three years might not be long enough to capture changes in plasma FAs over 20+ years of follow up usually observed in large cohorts. More importantly, this previous study did not assess the relation between single or repeated measures of FAs and outcome [10].

Therefore, the present study sought to assess variability of plasma phospholipid fatty acids on blood samples collected 15 years apart. In addition, we wished to determine whether the relation of omega-3 fatty acids with HF using a single baseline measurement of omega-3 fatty acids was comparable to results obtained using the average of two measurements of omega-3 fatty acids obtained at baseline and at about 15 years later during follow up among US male physicians.

Subjects and Methods

Study Population

We examined data from the Physicians' Health Study (PHS), a completed randomized, double blind, placebo-controlled trial designed to study low-dose aspirin and beta carotene for the primary prevention of cardiovascular disease and cancer in male physicians. A detailed description of the PHS has been previously published [11]. This ancillary study of the PHS used a prospective nested case-control design to evaluate the relation of plasma phospholipid omega-3 FA and HF. The design of the current ancillary study has been

previously described [4]. Briefly, to be eligible to be selected as a case, each participant must have provided two blood samples (baseline in 1982-83 and again between 1995 and 1997) and be free of HF at the time of the second blood collection. For each case, a risk set technique was used to randomly select a control among participants who provided two blood samples and were alive and free of HF at the time of index HF diagnosis. The controls were matched to cases on age (within 2 years), time of baseline blood collection within 289 days, year of birth within 2 years, and race. Each case was eligible to serve as a control prior to HF diagnosis. Similarly, each control was eligible to later become a HF case to assure that controls were representative of the total population that gave rise to the HF cases. Current analyses are based on 421 matched pairs, and participants were followed until 2009. Each participant gave written informed consent and the Institutional Review Board at Brigham and Women's Hospital approved the study protocol.

Ascertainment of HF in the PHS

HF in the PHS was ascertained by annual follow-up questionnaires. Specifically, a questionnaire was mailed to each participant every 6 months during the first year and was mailed annually thereafter to obtain information on compliance with the intervention and the occurrence of new medical diagnoses, including HF. Detailed description of HF validation in the PHS using review of medical records in a subsample has been published elsewhere [12, 13].

Measurement of plasma phospholipid FAs

Phospholipid fatty acids were measured in EDTA plasma, frozen at -70°C and stable during long-term storage, using the method previously described [14]. In summary, EDTA plasma was mixed with 0.9% saline and lipids were extracted from the plasma with a mixture of chloroform:methanol (2:1, v/v) under nitrogen, to prevent loss due to oxidation. Cholesterol esters, triglyceride and phospholipid subclasses were separated on silica gel TLC plates in a solvent mixture of petroleum ether, diethyl ether and glacial acetic acid (80:20:1, v/v/v). The phospholipid band was harvested for the formation of methyl esters. Fatty acid methyl esters were prepared with 14% boron trifluoride in methanol, incubated at 80°C for 90 minutes, and then extracted with petroleum ether. The final product was dissolved in heptane and injected onto a Varian CP7420 capillary 100m column using a Hewlett Packard 5890 gas chromatograph (GC) equipped with a HP6890A autosampler. The GC is configured for a single capillary column with a flame ionization detector. It is interfaced with HP chemstation software. Adequate separation of fatty acid methyl esters was obtained over 90 minutes with an initial temperature of 190°C for 25 min. The temperature was increased to 240°C at a rate of $2^{\circ}\text{C}/\text{min}$ and held for 5 minutes. Fatty acid methyl esters from 14:0 through 24:1n9 were separated, identified and quantities of 32 individual fatty acids were expressed as percent of total. Coefficients of variations obtained from controls were 5.3% for 18:3n3; 8.0% for 20:5n3; 3.8% for 22:5n3; and 5.9% for 22:6n3.

Other variables

Information on demographic variables, body mass index, cigarette smoking, exercise, alcohol consumption, and history of atrial fibrillation, hypertension, and hypercholesterolemia were collected at baseline.

Statistical Analysis

We computed Spearman correlation coefficients for omega-3 fatty acid measurement between 1982 and 1983 (hereon referred to as baseline measurement) and between 1995 and 1997 (subsequently referred to as follow up measurement) using the control series. To compute odds ratios (OR) with corresponding 95% confidence interval (CI) for baseline omega-3 FAs, we used conditional logistic regression with additional adjustment for body mass index (continuous), alcohol (never/past/current), smoking (rarely, monthly, weekly, daily), exercise (<weekly, 1-4/week, 5+/week), and history of atrial fibrillation and hypertension, and presented results as OR per one standard deviation (in controls) higher FAs. Because of missing data on smoking and alcohol history, 3 pairs were excluded from the final multivariable models. For hypertension and exercise, we created indicator variables for missing observations. This was repeated using the average and SD of baseline and follow up measurement in the models.

In sensitivity analysis, we additionally analyzed arachidonic acid (20:4n-6) and cis-palmitoleic fatty acid (16:1n-7). All analyses were performed by using SAS, version 9.2 (SAS Institute, Cary, NC). All p-values were two-tailed and significance level was set at an alpha of 0.05.

Results

Baseline characteristics of cases and controls are presented in Table 1. Means (SD) of omega-3 FAs measured at baseline and after 15 years of follow up in the control series are shown in Table 2. Spearman correlation coefficients between baseline and follow up plasma phospholipid fatty acids for ALA, EPA, DPA and DHA were 0.20, 0.45, 0.28, and 0.50, respectively, using the control series (Table 3).

In a conditional logistic regression model controlling for matching factors, body mass index, alcohol, smoking, exercise, and history of atrial fibrillation and hypertension, the estimated OR (95% CI) of HF per SD higher ALA (0.04) was 0.98 (0.85-1.13) when baseline measurement of ALA was used and 0.86 (0.74-1.01) when the average of baseline and follow up measurements was used. Corresponding values for total long chain omega-3 FAs (EPA + DHA + DPA) were 0.87 (0.73-1.03) and 0.88 (0.75-1.04) Table 4. In sensitivity analysis, spearman correlation coefficients between baseline and follow up values of arachidonic acid and cis-palmitoleic fatty acid were 0.64 and 0.36, respectively.

Discussion

In this cohort of US male physicians, we observed a modest correlation between individual plasma phospholipid fatty acids measured on blood samples collected 15 years apart. In addition, use of single measurement or mean FAs obtained on blood samples collected 15 years apart yielded similar conclusions on the relation of FAs with HF.

Measurement of plasma phospholipid FA profiles as a biomarker for dietary fat has been used in previous studies to estimate HF risk in the PHS [4, 15, 16], and in other cohorts [5, 6, 17]. However, because plasma phospholipid FAs have been shown to be moderately

correlated with dietary intake [18], the composition of FAs in plasma can change with dietary patterns and metabolism over time. As such, each of these studies is limited by the potential for exposure misclassification during follow-up. We found that plasma phospholipid omega-3 FAs measured during follow-up were slightly higher than baseline values. However, our findings on the relation of single vs. repeated measurement of FAs on HF risk suggest that a single measurement of FAs can be used to reasonably estimate the relation of FAs with HF.

Previous studies have attempted to measure potential exposure misclassification during follow-up. The Cardiovascular Health Study analyzing α -linolenic acid and incidence of congestive HF conducted a sensitivity analysis by restricting follow-up to 7 years to determine if a shorter follow-up time affected the results, and found no evidence in support of such hypothesis [17]. In a separate analysis, The Cardiovascular Health Study took a subsample of approximately 100 participants who had blood taken at baseline, 6 years, and 13 years in follow-up. The correlation coefficients between baseline and 13 year blood measurements were 0.50, 0.52, and 0.60 for EPA, DPA, and DHA, respectively [6]. These correlation coefficients appear comparable to those observed in our study. Of note is that CHS participants were older (mean age approximately 75 years) than PHS subjects who were in their fifties at baseline. In addition, a subset of 50 subjects of the Atherosclerosis Risk in Communities cohort with two blood samples collected at baseline and at 3 years reported correlation coefficients of 0.51 for EPA, 0.46 for DHA, and 0.35 for ALA [10], which are also comparable to values obtained in our study with about a 15 year window between the two measurements.

Strengths of our study include the prospective design of the PHS, the ability to control for residual confounding through collected covariates; standardized follow-up questionnaires to ascertain endpoints; a high positive predictive value (91%) of self-reported HF in male physicians [19]; and the availability of plasma phospholipid FA measurement 15 years after study inception. However, our study had several limitations. First, our study was an observational study and we cannot exclude residual or unmeasured confounding as a possible alternative explanation of our results. Second, our participants were male, mostly Caucasian physicians who may have different behaviors than the general population, thereby limiting the ability to generalize our findings. Third, we had limited statistical power to detect smaller effect size (85% power to detect odds ratio of 1.60 assuming two groups of exposure in a case-control design).

In conclusion, our data demonstrate a reasonable correlation between two assessments of FAs spaced by 15 years. In addition, when compared to repeated measurements, a single measurement of plasma phospholipid fatty acids appears appropriate to estimate the risk of HF over long-term follow up.

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Abbreviations

HF	heart failure
FA	fatty acid
PHS	Physicians' Health Study
ALA	α -linolenic acid
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid

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Table 1Characteristics of 421 heart failure cases and 421 matched controls in US male physicians¹

Characteristics	Cases (n=421)	Controls (n=421)
Age, y (mean ± SD)	58.2 ± 7.2	58.2 ± 7.2
BMI, kg/m ² (mean ± SD)	25.7±3.1	24.6 ± 2.3
History of atrial fibrillation (%)	6.2	1.7
History of CABG(%)	1.7	0.7
History of hypertension (%)	33.3	25.1
Current exercise (%)	73.8	75.5
Smoking status (%)		
Never	42.8	52.4
Past	47.3	39.8
Current	10.0	7.9
Alcohol (%)		
Rarely	14.3	14.3
Monthly	11.6	6.9
Weekly	47.7	47.5
Daily	26.4	31.3

BMI = Body Mass Index

CABG = Coronary artery bypass graft

¹ Few participants had missing data: hypertension (n=6), exercise (n=6), smoking (n=1), and alcohol use (n=2)

Table 2

Mean and standard deviation of baseline (1982-1983) and follow up (1995-1997) plasma fatty acid measurements in 421 controls and 421 cases

	Controls		Cases	
	1982-1983	1995-1997	1982-1983	1995-1997
Omega-3 fatty acids				
18:3n-3: α -Linolenic acid (ALA)	0.14 $\pm$ 0.04	0.19 $\pm$ 0.07*	0.14 $\pm$ 0.05	0.18 $\pm$ 0.06*
20:5n-3: Eicosapentaenoic acid (EPA)	0.76 $\pm$ 0.42	0.96 $\pm$ 0.54*	0.73 $\pm$ 0.35	0.90 $\pm$ 0.52*
22:5n-3: Docosapentaenoic acid (DPA)	0.84 $\pm$ 0.19	0.93 $\pm$ 0.20*	0.82 $\pm$ 0.22	0.90 $\pm$ 0.22*
22:6n-3: Docosahexaenoic acid (DHA)	3.09 $\pm$ 0.97	3.34 $\pm$ 1.12*	2.99 $\pm$ 0.89	3.28 $\pm$ 1.10*
20:5n-3 + 22:6n-3 (EPA+DHA)	3.85 $\pm$ 1.25	4.30 $\pm$ 1.50*	3.72 $\pm$ 1.12	4.18 $\pm$ 1.46*
20:5n-3 + 22:5n-3 + 22:6n-3 (EPA + DHA + DPA)	4.70 $\pm$ 1.33	5.24 $\pm$ 1.60*	4.53 $\pm$ 1.20	5.08 $\pm$ 1.56*
20:4n-6: Arachidonic acid (AA)	11.11 $\pm$ 1.71	10.39 $\pm$ 1.90*	11.19 $\pm$ 1.84	10.61 $\pm$ 2.02*
16:1n-7: Cis-Palmitoleic acid	0.31 $\pm$ 0.12	0.39 $\pm$ 0.17*	0.33 $\pm$ 0.14	0.39 $\pm$ 0.18*

% total fatty acids

*
p<0.0001 between 1982-1983 and 1995-1997

Table 3

Spearman correlation coefficients between baseline (1982-1983) and follow up (1995-1997) measurements in the control series

	Spearman correlation coefficients
Omega-3	
18:3n-3: α -Linolenic acid (ALA)	0.20 [*]
20:5n-3: Eicosapentaenoic acid (EPA)	0.45 [*]
22:5n-3: Docosapentaenoic acid (DPA)	0.28 [*]
22:6n-3: Docosahexaenoic acid (DHA)	0.50 [*]
20:5n-3 + 22:6n-3 (EPA+DHA)	0.49 [*]
20:5n-3 + 22:5n-3 + 22:6n-3 (EPA + DHA + DPA)	0.48 [*]
20:4n-6: Arachidonic acid (AA)	0.64 [*]
16:1n-7: Cis-Palmitoleic acid	0.36 [*]

^{*}
p<0.0001

Table 4

Odd ratio and 95% confidence interval of heart failure according to 1-standard deviation change of plasma omega-3 fatty acids^{1,2}

Omega-3 fatty acids	OR (95% CI) using baseline measure	OR (95% CI) using mean of baseline and follow up measure
Omega-3 fatty acids		
18:3n-3: α-Linolenic acid (ALA)	0.98(0.85-1.13)	0.86(0.74-1.01)
20:5n-3: Eicosapentaenoic acid (EPA)	0.90 (0.75-1.07)	0.86 (0.73-1.03)
22:5n-3: Docosapentaenoic acid (DPA)	0.85 (0.71-1.01)	0.81 (0.68-0.95)
22:6n-3: Docosahexaenoic acid (DHA)	0.90 (0.76-1.06)	0.93 (0.79-1.10)
20:5n-3 + 22:6n-3 (EPA+DHA)	0.88 (0.74-1.05)	0.90 (0.77-1.07)
20:5n-3 + 22:5n-3 + 22:6n-3 (EPA + DHA + DPA)	0.87 (0.73-1.03)	0.88 (0.75-1.04)
20:4n-6: Arachidonic acid (AA)	1.01 (0.87-1.17)	1.05 (0.91-1.21)
16:1n-7: Cis-Palmitoleic acid	1.12 (0.97-1.30)	1.08 (0.93-1.26)

¹ Conditional logistic regression adjusting for matching factors (age at randomization, year of birth, race, time of baseline blood collection, and randomization arm), body mass index, alcohol, smoking, exercise, and history of atrial fibrillation and hypertension

² Standard deviations for baseline measurement: ALA=0.04, EPA=0.42, DPA=0.19, DHA=0.97, EPA+DHA=1.25, EPA+DHA+DPA=1.33, AA=0.12, cis-palmitoleic =1.71; Standard deviation for the average of baseline and follow up measurement: ALA=0.04, EPA=0.40, DPA=0.15; DHA=0.90; EPA+DHA=1.19; EPA+DHA+DPA=1.26, AA=0.12, cis-palmitoleic = 1.63