Serum phospholipid fraction of polyunsaturated fatty acids is the preferred indicator for nutrition and health status in hemodialysis patients

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Abbreviations:

AA, arachidonic acid;

EC, endocannabinoids

FA, Fatty acids;

PL, phospholipids;

PUFA, polyunsaturated fatty acids;

TG, triacylglycerol;

Abstract

Long chain (LC) polyunsaturated fatty acids (PUFA) are major components of cell membrane phospholipids (PL) and serve as precursors for numerous bioactive lipid derivatives. Fatty acids (FA) are routinely analyzed in biological samples to assess composition of tissues, cells, and lipid fractions. In human studies, serum or plasma is often used because of their easy procurement. However, the lipid pool in serum and plasma is a mixture of triacylglycerol (TG), PL, cholesterol and its esters, and other components. Herein, we report findings from a serum FA analysis after fractionation of polar and neutral lipids by solid phase extraction in a large cohort of 400 hemodialysis patients. LC PUFA were found concentrated in the polar fraction compared to the total or the neutral lipid fraction. When correlated with clinical markers of disease, a greater number of significant correlations were found for PUFA in polar compared to total or the neutral fraction. We also observed that polar lipids are a reliable reflection of LC PUFA status compared to the total or neutral fractions because the latter are diluted by non-essential FA. The relative amounts of LC PUFA in the total and neutral fractions reflect the contribution of TG in blood that varies with diet, age, and physiologic state. Our data indicate that LC PUFA in the polar fraction are superior indicators of bioactive FA-status than in the total or the neutral fraction and should be used to establish important links between PUFA status, their bioactive substrates in hemodialysis patients.

Keywords: lipids, human, PUFA, serum, fatty acids, phospholipids, hemodialysis

1. Introduction

The study of fatty acid (FA) composition of tissue phospholipids (PL) is important because long chain (LC) polyunsaturated fatty acids (PUFA) associated with these lipids serve critical biological roles as the precursors for numerous bioactive lipid derivatives and in cell-to-cell communications[1, 2]. The PUFA-derived mediators include prostaglandins, leukotrienes, thromboxanes, endocannabinoids (EC), and other families of oxylipins. In human studies, serum or plasma is commonly used to evaluate fatty acid status because of its availability and the FA composition of circulating PL is the most reliable estimate of PUFA and essential fatty acid status PL[3].

Analyses of LC PUFA are routinely performed to assess dietary status and tissue composition related to their biological importance. The analysis begins with the extraction of lipids from the sample and in some cases isolation of lipid classes, for example, the polar lipids such as PL from those that are non-polar lipid compounds such as triacylglycerols (TG). Thus the blood lipid pool is a mixture of TG, PL, cholesterol and its esters, and other lipid constituents including FA. The FA composition of serum PL has become an acceptable indicator for assessing nutrition status and to predict dietary fat intakes and de novo lipid synthesis[4] but its importance in dialysis patients is not well established.

It is recognized that LC *n*-3 PUFA, by virtue of their biochemistry and numerous derivatives, participate in a number of key biological processes. An example are the oxylipin derivatives that participate in cell membrane physiology, cell membrane fluidity, cell signaling pathways, inflammation, and gene expression[1,3, 5-7]. The importance of LC *n*-3 PUFA to cardiovascular health is also of great scientific interest. With its anti-inflammatory, anti-arrhythmic, lipid-lowering, and anti-hypertensive effects, LC *n*-3 PUFA have been described in many reports to possess important cardioprotective benefits, especially for eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)[8-10]. This is especially relevant for the chronic hemodialysis population, which has exceptionally high rates of cardiovascular disease[11].

Higher amounts of PUFA are recognized to be associated with PL relative to the amounts found in neutral lipids like TG[12]. Because LC PUFA rarely accumulate in the neutral lipid fraction, only serum total lipids or polar LC PUFA will be useful as clinical biomarkers in hemodialysis subjects, a population with a large burden of illness[13]. Similar findings have been noted in non-dialysis patients[13]. Because LC PUFA in the polar lipid fraction is a better

and more stable reflection of total LC PUFA than what is found in other glycerol lipids such as TG, we firmly support that when studying the biological actions of PUFA the focus of attention should be on the polar lipid fraction. LC PUFA in the polar lipid fraction are subject to many biochemical pathways that liberate (such as by phospholipase A₂) PUFA for biosynthesis of potent biological mediators via the cyclooxygenase (COX), lipoxygenase (LOX), EC pathways and others[1, 2].

Therefore, the overarching hypothesis of this study is that the FA composition of serum polar lipid fraction best represents overall PUFA status in chronic hemodialysis patients and is therefore a better indicator of dietary status and well-being. The aims of this study were to confirm in hemodialysis patients that: 1) greater amounts of LC PUFA are found in the polar lipid fraction; 2) that LC PUFA in the polar fraction provide greater clinical relevance for essential FA status and dietary status of the subject or patient than other fractions; 3) that LC PUFA in the polar lipid fraction reflect more stable levels of LC PUFA than what is found in other glycerol lipids such as TG. The nutrition and clinical significance of our analyses is to achieve the best representative appraisal of PUFA to assess diet status and disease risk.

2. Subjects and Methods

2.1 Study population and collection of serum samples

The population of 10,044 individuals who participated in the Accelerated Mortality on Renal Replacement (ArMORR) project, a nationally representative prospective cohort study of patients who initiated chronic hemodialysis at one of 1,056 dialysis centers in the U.S, have been previously described[14]. This study was approved by the Institutional Review Board of the Massachusetts General Hospital, Boston, MA, ClinicalTrials.gov Identifier: NCT00505180.

2.2 Lipid class fractionation

Human serum samples were processed to determine FA composition in serum, and in neutral and polar lipid fractions after solid phase extraction. 100 µl was used for the total lipids analysis and 140 µl for the polar and neutral lipid fraction analyses. Briefly, serum lipids were extracted with 7.5 ml chloroform/methanol (2:1, vol/vol). For the analysis of fatty acids in the neutral and polar lipid fractions, solid phase extraction was used to fractionate the extracted lipids with chloroform

(20 ml) eluting the neutral lipid fraction and the methanol (20 ml) eluting the polar lipid fraction by a silica cartridge (300 mg filling, Alltech) attached to a 20 ml glass syringe[15, 16]. The fractions were then dried under nitrogen gas stream.

For the measurement of fatty acids in the total lipids and the neutral lipid fraction, the extracted lipids were treated with 0.5 ml 0.5 N NaOH in methanol at 100°C for 20 minutes, and fatty acid methyl esters (FAME) prepared by esterification using boron trifluoride (BF₃) in methanol (10% w/w, Supelco Inc. Bellefonte, PA) at 100°C using a heating block for 20 minutes in tightly sealed test tubes filled with nitrogen gas. The polar lipid fraction was methylated to FAME directly with 10% BF₃ in methanol after heating at 100°C for 10 minutes in a tightly sealed test tube after containing nitrogen gas. The resulting FAME was dissolved in 100 µl isooctane (HPLC grade, Fisher Scientific, Pittsburg, PA) and transferred to a GC sample vial with insert.

2.3 Gas chromatographic analysis of FAME

The resulting FAME from the total lipids, and the neutral and polar fractions of serum were analyzed by gas chromatography (GC) (HP 7890A series, autosampler 7693, GC ChemStation Rev.B.04.03, Agilent Technologies, Palo Alto, CA) with a DB-225 column (30 m, 0.25 mm i.d., 0.15 mm film thickness, Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector[15]. The oven temperature was programmed as following: initial temperature at 140°C, hold for 2 minutes, then ramp at 5°C/minute to reach a temperature of 180°C, hold for 12 minutes, then ramp at 15°C/minute to 210°C, hold for 11 minutes. Total GC run time was 35 minutes. Sample peaks were identified by comparison to authentic FAME standards (Nu-Chek-Prep Inc., Elysian, MN). Results of FAME analysis were obtained by weight percentage reports. Total LC *n*-3 PUFA were defined as the weight percentage of 20:5*n*-3 (EPA) + 22:5*n*-3 (docosapentaenoic acid) + 22:6*n*-3 (DHA), and total LC *n*-6 PUFA as the sum of the weight percentage of 20:4*n*-6 (arachidonic acid, AA) +22:4*n*-6+22:5*n*-6.

2.4 Statistical analyses

Data for serum FA composition were expressed as the means and standard deviations. The coefficient of variation (CV) was determined and the significance level between the deceased and the survived subject groups were calculated by the t-test procedure using SAS (version 9.3;

SAS Institute, Inc., Cary, NC, USA) and p values less than 0.05 were regarded as significantly different. Correlation analysis between serum FA and selected clinical parameters were performed by the Pearson correlation procedure in SAS and p values less than 0.05 were regarded as significantly different.

3. Results

The 400 ArMORR subjects used for this analysis were part of a separate study that included 100 deceased who died of sudden cardiac death during the first year on hemodialysis and 300 patients who survived (Supplemental Table 1). LC PUFA levels are higher in the polar fraction than in the total lipids (Table 1A) and neutral fraction (Table 1B). Levels of the two most common LC PUFA, AA and DHA, were consistently higher in the polar fraction compared to total lipids or neutral fraction, as were the total *n*-3 PUFA, *n*-3 LC PUFA, and *n*-6 LC PUFA. The total *n*-3 PUFA were higher in the polar (4.35%) compared to 2.71% in the total lipids and only 0.69% in the neutral lipid fraction. Total LC *n*-6 PUFA were also higher in the polar fraction at 11.41% compared to 8.09% in the total lipids and 5.26% in the neutral fraction. Total *n*-3 LC PUFA were 3.99% in the polar compared to 2.15% in the total lipids and merely 0.28% in the neutral lipid fractions. The ratio of LC *n*-6 PUFA to LC *n*-3 PUFA was lower in the polar lipid (3.06) compared to that of the total lipid (4.15) and neutral lipid fraction (8.30). Therefore, LC PUFA were found concentrated in the polar fraction compared to both the neutral fraction and the total lipids. The relative amounts of LC PUFA in the total lipids and neutral fraction reflect the contribution of TG in blood that varies with diet (amount and type of fat, caloric macronutrient intake), age (young versus old) and physiologic state (BMI and activity).

Our data also show that the polar lipid fraction is a more stable reflection of LC PUFA status compared to the other lipid fractions because the latter are diluted by non-essential FA. Specifically, Tables 2 and 3 show that smaller CV values for PUFA in the polar fraction were observed when compared to the total lipids and neutral lipid fraction (Table 2 for subjects who survived and Table 3 for those who died). Among all the FA measured in subjects who survived, AA of polar lipids had a CV value of 22.46 compared to 27.87 in the total lipids and 35.84 in the neutral fraction (Table 2). The CV for DHA was 34.05 in the polar fraction compared to 41.43 for the total lipids and 179.2 for the neutral fraction. For total PUFA, the CV of polar lipid was only 7.51 compared to 10.50 for the total lipids and 14.91 for the neutral lipid fraction.

Similarly, the CV for total lipid LC PUFA (POLY) was only 7.51 for the polar fraction while that of the total lipid was 10.50 and that for neutral lipid was 14.91 (Table 2). Likewise, the values for POLY for those who died of sudden cardiac death were 9.91, 13.87, and 19.03, respectively (Table 3). Of interest with respect to *n*-3 PUFA status and sudden cardiac death is that EPA and DHA levels in the polar lipid fractions were higher in hemodialysis patients, regardless of whether they survived or died of sudden cardiac death (Tables 2 and 3). Therefore, since these *n*-3 PUFA are believed to be lower in hemodialysis patients that puts them at risk for cardiovascular disease, a more precise measurement of their levels could be achieved by the polar lipid analysis of the PUFA. Furthermore, in the polar lipid fraction, DHA and total *n*-3 PUFA were higher in hemodialysis patients that were survivors. The smaller CV values observed in the polar lipid fraction for AA, DHA, and total LCPUFA indicates less variability for the polar lipid fraction, which potentially offers more predictive power when evaluating nutritional status and biomarker relationships for hemodialysis patients.

When comparing the difference in hemodialysis patient serum polar lipid fraction FA and clinical data between the deceased and the survived subjects, major LC PUFA species are noted to be significantly different between the two groups of subjects (Table 4). In particular the polar lipids revealed higher levels of DHA, total PUFA (POLY), total *n*-6 PUFA, total *n*-3 PUFA, and lower ratio of *n*-6 to *n*-3 PUFA in subjects who survived, while the total monounsaturated fatty acids were higher in those who died. The analysis of serum neutral lipid fractions FA values generally indicated no differences in PUFA or ratios of PUFA between hemodialysis subjects who survived or died (data not shown). Thus, there are distinctive differences between subjects who survived and died with regard to their serum PUFA composition for the polar lipid fraction.

A greater number of significant correlations with important clinical parameters such as blood pressure or blood cholesterol were found associated with primarily PUFA in the polar lipid fraction compared to the neutral lipid fraction or total lipids (Table 5). The values presented in Table 5 include the *p* values identified in brackets. Significant correlations between selected serum fatty acid values in the polar lipid fraction and clinical parameters were more frequently observed compared to the same analysis using the total lipid and neutral lipid fraction (32 in the polar fraction vs. 11 each in total lipid and 20 in neutral fraction). As shown in Table 5, more significant correlations of FA (primarily for PUFA) in the polar lipid fraction for clinical parameters were found when compared to the number of the significant correlations found in the

total lipid and neutral lipid fraction. The results show a robust relationship of higher PUFA in the chemical analysis of polar lipids compared to the FA analysis based on total lipid or neutral lipid fraction.

4. Discussion

In this investigation we found that in hemodialysis patients the polar fraction of serum lipids, which is rich in phospholipids, is preferentially enriched with LC PUFA [13] and is more strongly associated with some markers than found with the neutral fraction of PUFA. These observations coincide with the general population; even though the absolute LC n-3 PUFA content in hemodialysis patients is generally lower compared to the common population[13]. Recently, other investigators reported that the concentrations of saturated fatty acids in phospholipid of plasma were positively and n-6 PUFA inversely correlated to coronary heart disease risk[17]; while the concentration of trans linoleic acid in plasma phospholipids was associated with lowered risk of heart failure rate in a cohort of 788 matched pairs in the US male physicians' study[18]. It is firmly understood that the plasma polar lipid fraction contains the majority of PUFA present in the blood, which reflects the endogenous fatty acid metabolism (deacylation and reacylation of phospholipids) as well as dietary fatty acid intake of PUFA in an individual[19]. More importantly, PUFA content in circulating phospholipids approximates that of cell membrane phospholipids and thus, plasma/serum phospholipids may well represent the functional lipid pool[20]. Higher amounts of PUFA are indeed well recognized to be associated with the phospholipid fraction compared to the amounts found in neutral lipids like TG[12]. Another example to support our position is that AA has been shown to be highly enriched in the phospholipid (4%) fraction relative to the TG fraction (<1%)[12].

Herein we have demonstrated that LC PUFA are concentrated in the polar lipid fraction and the distribution of their values are in a stable, uniform range, implying greater value in predicting and assessing the essential fatty acid status in hemodialysis patients compared to the amounts found in the total lipid and the neutral lipid fraction. It is therefore logical to adopt the position that PUFA in the polar lipids possess greater biological and physiological significance compared to PUFA in other lipid fractions. For example, Holub et al. reported that phospholipid composition of serum or plasma is a valid biochemical marker for evaluating the status of

various fatty acids in human physiology[21]. LC PUFA are precursors of an entire spectra of biologically active compounds called oxylipins[1] and endocannabinoids[22]. When combined with an amide group, these PUFA will form the endocannabinoid class of lipid derivatives. Through the action of biochemical pathways that liberate LC PUFA in the PL fraction (such as by PLA₂), the LC PUFA can be further converted to biologically active compounds by the COX and LOX pathways to yield many potent biological mediators. In addition, it has been well established that phospholipids play important roles as lipids constituting the cell membrane and in plasma lipoproteins, and play critical roles in the mobilization of cholesterol from the cell membrane and the regulation of genetic functions and protein metabolism[23, 24]. The clinical significance of the n-3 LC PUFA in cardiac health is documented[25] but their role as precursors of oxylipins and inflammation is growing[1]. In addition to its application in lowering disease risk, defining LC n-3 PUFA content in blood is also important because it helps determine the risk level associated with the LC n-3 PUFA status in a particular individual or a population so that corresponding measures could be taken to adjust any imbalance in LC n-3 PUFA status. The connection between informative blood analysis of FA and cardiovascular diseases is supported by analysis of PL in polar lipids. Our premise is reinforced by the association of LC n-3 PUFA and the ratio of n-6/n-3 with hypertension [26] and diastolic blood pressure inversely related to monounsaturated fatty acids in 4680 adults [27]. Understanding the relationships of PUFA to disease should be based on the most accurate assessment of lipid class of blood samples for lipid analysis. In this case the polar lipids are not diluted with fatty acids of lower significance to the biomarkers that are most relevant to inflammation and blood pressure and diet assessment. Studies on oxylipins and endocannabinoids will require greater attention to specific lipid types such as phospholipids for linking these substrates to markers of human disease risk [1, 22].

Our results support the position that in hemodialysis patients, as in the general population, the polar lipid fraction of circulating fatty acids is the best fraction of fatty acid containing lipids with which to assess nutrition, health, and disease status. Further, as described, many clinical studies have relied on the fatty acid analysis of tissue phospholipids to determine dietary status or disease risk. Because of consistently higher levels of both AA and DHA, ratios of LC/SM, or other ratios that reflect overall status of LC PUFA, analysis of serum polar lipid LC PUFA could be the most useful indicators of general condition and disease risk. The polar lipid fraction is

important with regard to substrates for oxylipins and endocannabinoids associated with inflammation and systemic energy metabolism [1, 2, 22].

In summary, our data advocate that LC PUFA in the serum polar lipid fraction is a superior indicator of nutrition status and disease risk compared to total lipids or the neutral lipid fraction in the hemodialysis patient. Our study has some limitations, it was not possible to extensively investigate an exhaustive set of health and disease related clinical assessments with fatty acids in different lipid classes. Given our findings, this would be a logical approach in the dialysis patient. Furthermore, future study should also focus the work and resources on the polar fraction of blood lipids to produce and maximize meaningful findings for understanding endocannabinoids and appetite as well as oxylipins and inflammation. This new information can be used to measure and establish valid links between PUFA status for biomarkers to reduce disease risk in hemodialysis patients.

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Conflict of Interest

There is no conflict of interest to report.

Authorship

Allon Friedman and Bruce Watkins designed the experiment; Bruce Watkins drafted the manuscript; Hector Tamez, Julia Wenger, Ravi Thadhani provided the samples used in the research. Analyses were performed in the Advance Technology Laboratory, Nutrition and Molecular BioSciences, University of Connecticut.

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Table 1. PUFA serum values in the polar lipid compared to the total lipid and neutral lipid fraction of all subjects

A: Polar vs. total lipids

Fatty acids	Polar (n = 400)			Total (n = 400)			T-test p value
	Mean	SD	CV	Mean	SD	CV	
18: 2 <i>n</i> -6	18.65	2.78	15	28.50	4.06	14	<.0001
20: 4 <i>n</i> -6	10.48	2.48	24	7.60	2.20	29	<.0001
22: 6 <i>n</i> -3	2.87	1.10	38	1.43	0.65	45	<.0001
TN6	32.41	2.67	8	38.23	4.45	12	<.0001
TN3	4.35	1.43	33	2.71	0.97	36	<.0001
TN6/TN3	8.09	2.28	28	15.58	5.74	37	<.0001
N6LC	11.41	2.61	23	8.09	2.30	28	<.0001
N3LC	3.99	1.45	36	2.15	0.96	45	<.0001
LCN6/N3	3.06	0.80	26	4.15	1.55	37	<.0001
LCPUFA	15.39	3.46	22	10.24	2.85	28	<.0001
LC/SM	0.26	0.07	26	0.18	0.06	32	<.0001
ED/SM	0.05	0.02	45	0.03	0.02	54	<.0001
AA/SM	0.17	0.05	26	0.14	0.05	33	<.0001

B: Polar vs. neutral fraction

Fatty acids	Polar (n = 400)			Neutral (n = 400)			T-test p value
	Mean	SD	CV	Mean	SD	CV	
18: 2 <i>n</i> -6	18.65	2.78	15	33.42	5.40	16	<.0001
20: 4 <i>n</i> -6	10.48	2.48	24	5.25	1.96	37	<.0001
22: 6 <i>n</i> -3	2.87	1.10	38	0.21	0.40	191	<.0001
TN6	32.41	2.67	8	38.74	6.34	16	<.0001
TN3	4.35	1.43	33	0.69	0.85	124	<.0001
TN6/TN3	8.09	2.28	28	40.19	17.29	43	<.0001
N6LC	11.41	2.61	23	5.26	1.96	37	<.0001
N3LC	3.99	1.45	36	0.28	0.64	230	<.0001
LCN6/N3	3.06	0.80	26	8.30	3.69	45	<.0001
LCPUFA	15.39	3.46	22	5.53	2.11	38	<.0001
LC/SM	0.26	0.07	26	0.10	0.05	45	<.0001
ED/SM	0.05	0.02	45	0.01	0.01	232	<.0001
AA/SM	0.17	0.05	26	0.10	0.04	44	<.0001

Abbreviations: CV, coefficient of variation; AA, 20:4n-6; TOTS, total saturated; TOTM, total monounsaturated; POLY, total polyunsaturated; TN6, total *n*-6 PUFA; TN3, total *n*-3 PUFA; N6LC, total long-chain *n*-6 PUFA (20C and 22C); N3LC, total long-chain *n*-3 PUFA (20C and 22C); LCPUFA, the sum of N6LC and N3LC; LC/SM, LCPUFA/(TOTS + TOTM); ED/SM, (EPA + DHA)/(TOTS + TOTM); AA/SM, AA/(TOTS + TOTM).

Table 2. Serum fatty acid composition (wt %) in hemodialysis survivors

Fatty acids	Total lipids (n = 300)			Neutral lipids (n = 300)			Polar lipids (n = 300)		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
14: 0	0.50	0.31	61.99	0.46	0.55	118.2	0.27	0.18	67.37
14: 1 <i>n</i> -5	0.0002	0.003	1732	ND			ND		
15: 0	0.11	0.09	82.97	ND			0.02	0.05	247.9
16: 0	20.13	1.79	8.880	17.84	2.17	12.14	22.25	1.98	8.880
<i>trans</i> -16: 1	0.52	0.14	26.55	ND			0.24	0.14	60.39
16: 1 <i>n</i> -7	1.64	0.60	36.50	2.24	0.86	38.44	0.76	0.41	54.35
17: 0	0.27	0.07	25.67	0.01	0.05	578.1	0.37	0.07	17.85
18: 0	6.89	1.34	19.47	4.56	2.39	52.43	17.57	2.56	14.56
18: 1 <i>n</i> -9	23.80	3.08	12.96	28.39	3.88	13.65	15.56	3.25	20.91
18: 1 <i>n</i> -7	2.34	0.42	17.81	2.11	0.48	22.92	2.37	0.34	14.24
18: 2 <i>n</i> -6	28.65	3.88	13.56	33.67	5.15	15.28	18.66	2.80	14.98
18: 3 <i>n</i> -6	0.34	0.21	61.36	ND			0.03	0.08	250.8
18: 3 <i>n</i> -3	0.57	0.23	40.90	0.44	0.47	107.4	0.37	0.22	59.35
20: 0	0.004	0.02	504.9	0.0009	0.02	1732	0.03	0.06	247.3
20: 1 <i>n</i> -9	0.12	0.12	93.22	0.01	0.10	832.8	0.10	0.12	122.8
20: 2 <i>n</i> -6	0.33	0.11	34.53	0.01	0.05	762.7	0.47	0.10	21.56
20: 3 <i>n</i> -6	0.97	0.43	44.92	0.06	0.23	366.6	1.86	0.77	41.62
20: 4 <i>n</i> -6	7.81	2.17	27.87	5.39	1.93	35.84	10.81	2.43	22.46
20: 5 <i>n</i> -3	0.33	0.29	89.60	0.08	0.28	367.1	0.37	0.36	96.74
22: 0	ND			0.002	0.03	1732	0.16	0.17	105.2
22: 1 <i>n</i> -9	ND			ND			0.04	0.11	234.4
22: 4 <i>n</i> -6	0.28	0.08	29.42	0.004	0.04	1178	0.49	0.12	23.70
22: 5 <i>n</i> -6	0.23	0.11	49.73	ND			0.45	0.16	35.72
22: 5 <i>n</i> -3	0.42	0.12	29.28	ND			0.79	0.19	24.51
22: 6 <i>n</i> -3	1.50	0.62	41.43	0.22	0.40	179.2	3.01	1.02	34.05
24: 0	0.02	0.05	249.8	ND			0.08	0.13	151.3
24: 1 <i>n</i> -9	0.09	0.09	101.7	ND			0.27	0.22	82.25
TOTS	27.91	2.44	8.750	22.87	3.77	16.49	40.76	2.84	6.960
TOTM	28.51	3.54	12.40	32.76	4.34	13.23	19.34	3.78	19.52
POLY	41.40	4.35	10.50	39.87	5.95	14.91	37.29	2.80	7.510
TN6	38.59	4.08	10.58	39.14	5.96	15.24	32.76	2.45	7.490
TN3	2.82	0.90	31.83	0.73	0.83	113.0	4.53	1.31	28.87
N6LC	8.31	2.27	27.27	5.40	1.93	35.73	11.74	2.55	21.71
N3LC	2.25	0.90	40.04	0.30	0.61	206.1	4.16	1.33	32.06
LCPUFA	10.56	2.79	26.40	5.70	2.08	36.48	15.90	3.30	20.74
LC/SM	0.19	0.06	30.41	0.11	0.05	43.22	0.27	0.06	23.41
ED/SM	0.03	0.02	49.01	0.01	0.01	210.3	0.06	0.02	39.25
AA/SM	0.14	0.04	31.69	0.10	0.04	42.56	0.18	0.04	24.82

Abbreviations: AA, 20:4*n*-6; TOTS, total saturated; TOTM, total monounsaturated; POLY, total polyunsaturated; TN6, total *n*-6 PUFA; TN3, total *n*-3 PUFA; N6LC, total long-chain *n*-6 PUFA (20C and 22C); N3LC, total long-chain *n*-3 PUFA (20C and 22C); LCPUFA, the sum of N6LC and N3LC; LC/SM, LCPUFA/(TOTS + TOTM); ED/SM, (EPA + DHA)/(TOTS + TOTM); AA/SM, AA/(TOTS + TOTM); ND, not detected, below detection limit of 10 ng/peak.

Table 3. Serum fatty acid composition (wt %) in hemodialysis patients who died of sudden cardiac death

Fatty acids	Total lipids (n = 100)			Neutral lipids (n = 100)			Polar lipids (n = 100)		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
14: 0	0.52	0.33	63.34	0.41	0.60	146.3	0.31	0.21	66.80
14: 1 <i>n</i> -5	0.001	0.01	1000	0.002	0.02	1000	ND		
15: 0	0.09	0.10	110.2	ND			0.03	0.06	229.6
16: 0	20.73	2.08	10.03	18.31	2.43	13.28	22.89	2.31	10.07
<i>trans</i> -16: 1	0.52	0.14	27.86	ND			0.25	0.15	59.70
16: 1 <i>n</i> -7	1.75	0.72	41.32	2.33	1.04	44.67	0.85	0.42	49.06
17: 0	0.26	0.10	39.99	0.02	0.08	456.2	0.37	0.07	18.00
18: 0	6.91	1.37	19.82	4.99	2.41	48.26	17.32	2.39	13.82
18: 1 <i>n</i> -9	24.89	3.81	15.32	29.24	4.34	14.84	16.99	3.65	21.49
18: 1 <i>n</i> -7	2.39	0.33	13.70	2.03	0.66	32.70	2.39	0.34	14.06
18: 2 <i>n</i> -6	28.08	4.55	16.19	32.67	6.05	18.52	18.63	2.76	14.84
18: 3 <i>n</i> -6	0.33	0.25	76.33	ND			0.04	0.10	249.3
18: 3 <i>n</i> -3	0.53	0.23	44.35	0.34	0.43	125.9	0.35	0.23	64.68
20: 0	0.01	0.02	420.3	ND			0.02	0.06	306.7
20: 1 <i>n</i> -9	0.13	0.11	88.46	0.02	0.08	478.7	0.11	0.13	114.9
20: 2 <i>n</i> -6	0.36	0.17	47.61	0.01	0.06	574.2	0.48	0.12	24.44
20: 3 <i>n</i> -6	0.94	0.45	47.85	0.05	0.14	298.4	1.84	0.73	39.68
20: 4 <i>n</i> -6	6.99	2.16	30.88	4.83	1.99	41.14	9.48	2.37	25.03
20: 5 <i>n</i> -3	0.28	0.38	135.6	0.05	0.36	755.4	0.31	0.47	150.7
22: 0	ND			ND			0.14	0.17	114.1
22: 1 <i>n</i> -9	ND			0.003	0.03	1000	0.05	0.10	199.3
22: 4 <i>n</i> -6	0.26	0.11	44.21	ND			0.49	0.13	26.06
22: 5 <i>n</i> -6	0.20	0.13	64.27	ND			0.42	0.17	40.19
22: 5 <i>n</i> -3	0.35	0.16	44.89	ND			0.69	0.18	25.74
22: 6 <i>n</i> -3	1.22	0.67	55.08	0.16	0.39	240.3	2.47	1.21	49.16
24: 0	0.02	0.04	244.7	ND			0.07	0.13	186.4
24: 1 <i>n</i> -9	0.08	0.10	117.8	ND			0.30	0.27	91.59
TOTS	28.53	2.94	10.32	23.72	4.19	17.68	41.15	3.34	8.120
TOTM	29.75	4.31	14.50	33.62	4.66	13.87	20.93	4.18	19.96
POLY	39.53	5.48	13.87	38.11	7.25	19.03	35.21	3.49	9.910
TN6	37.15	5.29	14.23	37.55	7.26	19.33	31.38	3.00	9.580
TN3	2.38	1.10	46.16	0.55	0.91	163.7	3.83	1.65	43.06
N6LC	7.45	2.29	30.71	4.83	1.99	41.14	10.39	2.55	24.49
N3LC	1.86	1.07	57.85	0.21	0.70	331.6	3.48	1.67	48.07
LCPUFA	9.30	2.85	30.61	5.04	2.14	42.57	13.87	3.49	25.16
LC/SM	0.16	0.06	36.23	0.09	0.05	50.99	0.23	0.07	29.07
ED/SM	0.03	0.02	67.86	0.004	0.01	323.7	0.05	0.03	61.06
AA/SM	0.12	0.05	36.77	0.09	0.04	49.79	0.15	0.04	28.50

Abbreviations: AA, 20:4*n*-6; TOTS, total saturated; TOTM, total monounsaturated; POLY, total polyunsaturated; TN6, total *n*-6 PUFA; TN3, total *n*-3 PUFA; N6LC, total long-chain *n*-6 PUFA (20C and 22C); N3LC, total long-chain *n*-3 PUFA (20C and 22C); LCPUFA, the sum of N6LC and N3LC; LC/SM, LCPUFA/(TOTS + TOTM); ED/SM, (EPA + DHA)/(TOTS + TOTM); AA/SM, AA/(TOTS + TOTM); ND, not detected, below detection limit of 10 ng/peak.

Table 4. Relationship between clinical parameters and fatty acids in serum polar lipid fraction

measurements	Survived (n = 300)		Deceased (n = 100)		T-test p value
	Mean	SD	Mean	SD	
BMI	26.43	6.77	25.45	6.73	0.21
SBP	144	21	139	27	0.074
DBP	73	12	72	14	0.62
CHOL	152	44	147	52	0.5
18: 2 <i>n</i> -6	18.66	2.80	18.63	2.76	0.92
20: 4 <i>n</i> -6	10.81	2.43	9.48	2.37	<.0001
20: 5 <i>n</i> -3	0.37	0.36	0.31	0.47	0.27
22: 6 <i>n</i> -3	3.01	1.02	2.47	1.21	0.0001
TOTS	40.76	2.84	41.15	3.34	0.29
TOTM	19.34	3.78	20.93	4.18	0.004
POLY	37.29	2.80	35.21	3.49	<.0001
TN6	32.76	2.45	31.38	3.00	<.0001
TN3	4.53	1.31	3.83	1.65	0.0002
TN6/TN3	7.76	2.04	9.08	2.67	<.0001
N6LC	11.74	2.55	10.39	2.55	<.0001
N3LC	4.16	1.33	3.48	1.67	0.0003
LCn6/LCn3	2.99	0.75	3.26	0.90	0.0065
LCPUFA	15.90	3.30	13.87	3.49	<.0001
LC/SM	0.27	0.06	0.23	0.07	<.0001
ED/SM	0.06	0.02	0.05	0.03	0.0042
AA/SM	0.18	0.04	0.15	0.04	<.0001

Abbreviations: TOTS, total saturated; TOTM, total monounsaturated; POLY, total polyunsaturated; TN6, total *n*-6 PUFA; TN3, total *n*-3 PUFA; N6LC, total long-chain *n*-6 PUFA (20C and 22C); N3LC, total long-chain *n*-3 PUFA (20C and 22C); LCPUFA, the sum of N6LC and N3LC; LC/SM, LCPUFA/(TOTS + TOTM); ED/SM, (EPA + DHA)/(TOTS + TOTM); AA/SM, AA/(TOTS + TOTM).

BMI: body mass index.

SBP: Average of pre-treatment sitting systolic blood pressures

DBP: Average of pre-treatment sitting diastolic blood pressures

CHOL: baseline cholesterol

Fatty acids and ratios are for polar lipids FAME.

Table 5. Correlations between fatty acids in serum total lipid, polar lipid or neutral lipid and blood pressure (SBP and DBP) and cholesterol levels

Fatty acids	Correlation coefficient (r)								
	SBP (n = 400)			DBP (n = 400)			Cholesterol (n = 272)		
	Total	Polar	Neutral	Total	Polar	Neutral	Total	Polar	Neutral
18: 2 <i>n</i> -6							0.232 [0.0001]	0.177 [0.003]	0.204 [0.0007]
20: 4 <i>n</i> -6		0.110 [0.028]			0.114 [0.023]				
20: 5 <i>n</i> -3	0.115 [0.022]	0.111 [0.027]	0.102 [0.042]				0.120 [0.047]	0.137 [0.024]	0.165 [0.0065]
22: 6 <i>n</i> -3		0.135 [0.007]	0.122 [0.015]					0.200 [0.0009]	0.352 [<.0001]
TOTS								-0.158 [0.009]	
TOTM		-0.105 [0.036]	-0.103 [0.039]				-0.209 [0.0005]	-0.144 [0.017]	-0.184 [0.0023]
POLY		0.135 [0.007]	0.102 [0.042]				0.181 [0.003]	0.284 [<.0001]	0.245 [<.0001]
TN6							0.16608 [0.006]	0.217 [0.0003]	0.196 [0.0012]
TN3	0.113 [0.024]	0.158 [0.002]	0.152 [0.002]				0.120 [0.047]	0.231 [0.0001]	0.388 [<.0001]
TN6/TN3		-0.142 [0.005]						-0.182 [0.003]	
N6LC		0.107 [0.032]			0.117 [0.020]				
N3LC	0.103 [0.039]	0.151 [0.003]	0.124 [0.013]					0.213 [0.0004]	0.302 [<.0001]
LCn6/LCn3								-0.187 [0.002]	
LCPUFA		0.145 [0.004]	0.118 [0.018]		0.125 [0.013]			0.124 [0.040]	0.142 [0.019]
LC/SM		0.156 [0.002]	0.108 [0.031]		0.128 [0.010]			0.168 [0.006]	0.158 [0.009]
ED/SM	0.099 [0.048]	0.150 [0.003]	0.119 [0.017]				0.124 [0.041]	0.225 [0.0002]	0.311 [<.0001]
AA/SM		0.124 [0.013]			0.119 [0.017]				
# of p < 0.05	4	13	9	0	5	0	7	14	11

Data in the row following the fatty acid are correlation coefficient (r) and the numbers in brackets in the lower row below the correlation coefficient are p values.

Only significant values are shown, all non-significant values are not shown.

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure, TOTS, total saturated; TOTM, total monounsaturated; POLY, total polyunsaturated; TN6, total *n*-6 PUFA; TN3, total *n*-3 PUFA; N6LC, total long-chain *n*-6 PUFA (20C and 22C); N3LC, total long-chain *n*-3 PUFA (20C and 22C); LCPUFA, the sum of N6LC and N3LC; LC/SM, LCPUFA/(TOTS + TOTM); ED/SM, (EPA + DHA)/(TOTS + TOTM); AA/SM, AA/(TOTS + TOTM).