Serial circulating omega 3 polyunsaturated fatty acids and healthy ageing among older adults in the Cardiovascular Health Study: prospective cohort study

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ABSTRACT

OBJECTIVE

To determine the longitudinal association between serial biomarker measures of circulating omega 3 polyunsaturated fatty acid (n3-PUFA) levels and healthy ageing.

DESIGN

Prospective cohort study.

SETTING

Four communities in the United States (Cardiovascular Health Study) from 1992 to 2015.

PARTICIPANTS

2622 adults with a mean (SD) age of 74.4 (4.8) and with successful healthy ageing at baseline in 1992-93.

EXPOSURE

Cumulative levels of plasma phospholipid n3-PUFAs were measured using gas chromatography in 1992-93, 1998-99, and 2005-06, expressed as percentage of total fatty acids, including α-linolenic acid from plants and eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid from seafoood.

MAIN OUTCOME MEASURE

Healthy ageing defined as survival without chronic diseases (ie, cardiovascular disease, cancer, lung disease, and severe chronic kidney disease), the absence of cognitive and physical dysfunction, or death from other causes not part of the healthy ageing outcome after age 65. Events were centrally adjudicated or determined from medical records and diagnostic tests.

RESULTS

Higher levels of long chain n3-PUFAs were associated with an 18% lower risk (95% confidence interval 7% to 28%) of unhealthy ageing per interquintile range after multivariable adjustments with time-varying exposure and covariates. Individually, higher eicosapentaenoic acid and docosapentaenoic acid (but not docosahexaenoic acid) levels were associated with a lower risk: 15% (6% to 23%) and 16% (6% to 25%), respectively. α-linolenic acid from plants was not noticeably associated with unhealthy ageing (hazard ratio 0.92, 95% confidence interval 0.83 to 1.02).

CONCLUSIONS

In older adults, a higher cumulative level of serially measured circulating n3-PUFAs from seafood (eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid), eicosapentaenoic acid, and docosapentaenoic acid (but not docosahexaenoic acid from seafood or α-linolenic acid from plants) was associated with a higher likelihood of healthy ageing. These findings support guidelines for increased dietary consumption of n3-PUFAs in older adults.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Healthy ageing is generally defined as survival without chronic disease or cognitive and physical dysfunction

Omega 3 polyunsaturated fatty acids (n3-PUFAs) from seafood and plants exert favorable physiologic effects that could benefit healthy ageing

Higher self reported estimates of dietary n3-PUFA and baseline biomarker n3-PUFA levels are each inversely associated with the risk of cardiovascular disease

WHAT THIS STUDY ADDS

Higher levels of serially measured circulating n3-PUFAs from seafood were associated with a lower risk of unhealthy ageing after multivariable adjustments

Increased dietary consumption of n3-PUFAs in older adults is recommended

Introduction

With rapid ageing of populations globally and an increased prevalence of chronic disease,[1 2] focus on longevity alone is shifting toward an emphasis on healthy ageing.[3] In contrast with years of total life, healthy ageing can be considered as living a meaningful lifespan without chronic diseases and with intact physical and mental function.[4 5] This measure builds on the concept of positive deviance,[6] identifying people resistant to failure amongst their peers, and more importantly, new modifiable determinants of this resistance.

In short term trials, omega 3 polyunsaturated fatty acids (n3-PUFAs) from seafood and plants exert many favorable molecular and physiologic effects that could promote aspects of healthy ageing.[7-10] Conversely, when links between n3-PUFAs and individual component outcomes that help define healthy ageing have been studied, conflicting relations have been reported. For example, higher self reported estimates of dietary n3-PUFA and biomarker n3-PUFA levels are each inversely associated with the risk of cardiovascular disease,[11-13] fatal coronary heart disease in particular,[12] but can be associated with higher risk of prostate cancer,[14 15] with mixed or inconclusive findings for dietary or biomarker n3-PUFA levels and cancer,[14-23] diabetes, lung disease, chronic kidney disease, and cognitive and physical function. Thus, the relation of n3-PUFAs to overall healthy ageing in older adult populations is not well established.

To investigate n3-PUFA intake and health outcomes, most studies have relied on self reported dietary questionnaires.[11 13 14 16 17 19-22] Few studies have used biomarkers,[12] which provide a complementary measurement to self report with less recall bias and estimation errors. In addition, biomarkers greatly facilitate the investigation of effects of individual n3-PUFAs. The n3-PUFAs include long chain eicosapentaenoic acid and docosahexaenoic acid from seafood, and docosapentaenoic acid that has been endogenously metabolized (to a lesser extent, also sourced from seafood); and also the essential α-linolenic acid from plants. Additionally, all previous biomarker studies have used one measure of n3-PUFA at baseline, which do not account for trends or changes over time. Serial measures over many years are required to overcome this limitation. To address these gaps in knowledge, we used serial measures of n3-PUFA biomarkers in the Cardiovascular Health Study to investigate the association between circulating phospholipid n3-PUFA levels and the likelihood of healthy ageing.[12 15] We hypothesize that higher cumulative levels of serially measured n3-PUFAs, especially long chain n3-PUFAs from seafood will be associated with a greater likelihood of healthy ageing.

Methods

Study design and population

The Cardiovascular Health Study is a multicenter prospective cohort of older adults in the United States.[24] This cohort is optimal for exploring factors that can help lengthen survival free of disability and improve quality of later life. In 1989-90, 5201 ambulatory adults who were living independently and were not under active treatment for cancer were recruited from a random sample of Medicare eligibility rolls (national public health insurance program) from four communities in the US (Sacramento County, CA; Washington County, MD; Forsyth County, NC; and Pittsburgh, PA).[25] An additional 687 African-American participants were recruited similarly in 1992-93. Thus, a total of 5888 participants were recruited. Trained personnel performed annual clinic examinations through 1999 to assess demographics, health status, hospital stays, medical history, and lifestyle.[26 27] Vital status follow-up was nearly 100% complete; less than 1% of all person time was otherwise missing and censored early. In addition, semiannual phone interviews were conducted continuously from enrolment through June 2015 to ascertain health status and incident cardiovascular disease events. In 2005-06, the remaining participants (n=1674) were evaluated in person or by phone to reassess cognitive and physical function.[28] All protocols were approved at the institutional review board of each participating university. For this analysis starting in 1992-93, after we excluded participants deceased before this study visit (n=438), without available blood measurements owing to not giving consent for the blood draw, depleted stored blood samples, or less likely, not attending the clinic appointment (n=891, 16.3% of living participants), who reported baseline fish oil supplements (to avoid reverse causality; n=195), and with prevalent unhealthy ageing in 1992-93 (defined in table 1; n=1742), for a total of 2622 participants eligible for analysis.

Circulating fatty acid measurements

Forty six distinct plasma phospholipid n3-PUFA levels were measured, as weight percentage of total fatty

Table 1 | The definition of healthy ageing* and its components

Components	Method of ascertainment
Cardiovascular disease	
1989-2015	Myocardial infarction, congestive heart failure, stroke, transient ischaemic attack, or claudication based on centralized adjudicated events for the entire follow-up period†.
Cancer	
1989-2015	Defined by 2015 National Institute of Health, Surveillance, Epidemiology, and End Results casefinding list, diagnosed from hospital stays, including secondary diagnosis for the entire duration of follow-up.
Lung disease (Chronic obstructive pulmonary disease)	
1989-2015	Defined as two or more diagnoses of chronic bronchitis (code 491), emphysema (code 492), bronchiectasis (code 494), or chronic airway obstruction (code 496) from any hospital stay, including secondary diagnosis identified for the entire duration of follow-up.
1989-90, 1993-94, and 1996-97	Spirometry forced vital capacity to forced expiratory volume (FEV) ratio less than 70 and FEV_1 predicted value less than 50.
Severe chronic kidney disease	
1989-90, 1992-93, and 1996-97	Defined as creatinine-cystatin C-based estimated glomerular filtration rate less than 10.[69-71]
1989-2015	Hemodialysis (procedure 39.95), peritoneal dialysis (procedure 54.98), postsurgical renal dialysis status (V45.1), and encounter for dialysis and dialysis catheter care (V56.1-V56.32) from any hospital stay, including secondary diagnosis.[69-71]
Physical function	
1989-1999 and 2005-2015	Activities of Daily Living, defined by persistent reported difficulties on two consecutive visits with ≥1 daily living in six domains, namely, eating, bathing, toileting, dressing, getting out of bed or chair and walking around at home, or one report regarding subsequent death or missing follow-up. Event defined by the first record of difficulty.
Cognitive function	
1990-1999 and 2005-2015	A modified Mini Mental State Examination score (MMSE) of 80 or less,[72] if unavailable, estimated from Telephone Interview for Cognitive Status, Informant Questionnaire on Cognitive Decline in the Elderly on two consecutive visits, or on one visit with no follow-up.[73] Event defined by the first record of a low MMSE score.
Death	
1989-2015	Death owing to cardiovascular disease, cancer, or lung disease are ascertained throughout follow-up. Deaths owing to other causes are censored.

*The healthy ageing definition is based on previous established definition in Cardiovascular Health Study (supplementary materials, table A).[46 74]
†All events were reviewed and classified as described in a previous publication.[75]

acids, at the Fred Hutchinson Cancer Research Centre Biomarker Laboratory, in stored plasma specimens collected in 1992-93, 1998-99, and 2005-06. At each collection, 12-hour fasting blood samples were collected and stored at −80°C, at which n3-PUFA levels remain stable in long term storage including after multiple freeze-thaw cycles.[29 30] Total lipids were extracted from plasma and phospholipids separated from neutral lipids by using one-dimensional thin layer chromatography.[31 32] Fatty acid methyl esters were derived from direct transesterification of phospholipid fractions and separated by gas chromatography.[33 34] Identification, precision, and accuracy were evaluated throughout the analysis by using model mixtures of known fatty acid methyl esters and established in house controls. Identification of fatty acid methyl esters was confirmed by gas chromatography-mass spectrometry at the US Department of Agriculture.[35] Laboratory coefficients of variations were less than 4% for each n3-PUFA.

Other risk factors

Sociodemographic information included age (years), sex (male or female), ethnicity (white or non-white), enrolment site, education (<high school, high school, some college, or college graduate), and income (<$12 000, $12 000-$24 999, $25 000-$49 999, or >$50 000/year; $1.00=£0.77=€0.87). Other risk factors were assessed at each visit using standardized procedures, including anthropometrics (height and weight to calculate body mass index (in kg/m^2), and waist circumference (cm)), physical activity excluding chores (<500, 500-1000, 1000-1500, or >1500 kcal/week; 1kcal=4.18kJ), blood pressure (mm Hg), high-sensitivity C-reactive protein (in categories of <9.5, 9.5-28.6, or >28.6 nmol/L), low density lipoprotein (mmol/L), high density lipoprotein (mmol/L), and triglycerides (mmol/L).[24 26 27 36 37] Information was collected on smoking status (never, former, or current), self perceived general health (excellent or very good; good; or fair or poor), family history of myocardial infarction or stroke, or both (yes or no), hypertension drugs (yes or no), lipid lowering drugs (yes or no), prevalent diabetes (yes or no, ascertained as specified in supplementary materials, table A), self reported depression (score of 1 to 10 based on the 10 item Centers for Epidemiological Studies' Depression Scale),[38] prevalent osteoporosis (yes or no), and treated arthritis (yes or no). Alcohol use (wine, beer, or liquor; reported as 0, 0-0.5, 0.5-1, 1-2, 3-7, 8-14, or >14 servings/week) was assessed at each visit using standardized questionnaires.[39] Dietary habits were assessed in 1989-90 by using a 99 item validated food frequency questionnaire and in 1995-96 by using a 131 item validated food frequency questionnaire,[27 40-42] including energy intake (kcal/day), fruit intake (servings/day), vegetable intake (servings/day), non-processed red meat intake (servings/day), processed meat intake (servings/day), total fish intake (servings/week), wholegrain intake (g/day), and dietary fiber intake (g/day). Dietary fiber intake was estimated using the Association of Official Agricultural Chemists Official Method 991.43, Total, Soluble, and Insoluble Dietary Fibers in Foods. Correlations between dietary fish intake and n3-PUFA biomarkers at baseline were evaluated in a previous publication, where eicosapentaenoic acid and docosahexaenoic acid levels were moderately correlated with tuna and other fish intake (r=0.55).[43] Other fatty acid biomarkers including omega 6 polyunsaturated fatty acids (n6-PUFAs) (including the sum of linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, and arachidonic acid), and *trans* fatty acids (including the sum of *trans*-16:1 (*trans*-16:1n7, *trans*-16:1n9), total *trans*-18:1 (including the sum of isomers *trans*-18:1n6, *trans*-18:1n7, *trans*-18:1n8, *trans*-18:1n9, and *trans*-18:1n10-12), and total *trans*-18:2 (including the sum of isomers *trans,trans*-18:2n6, *cis,trans*-18:2n6, and *trans,cis*-18:2n6)) were quantified simultaneously with n3-PUFAs as weight percentage of total fatty acids, described above. Apolipoprotein E genotype (ε2/ε2, ε2/ε3, and ε3/ε3 as the reference for ε4 allele non-carriers; ε2/ε4, ε3/ε4, and ε4/ε4 as ε4 allele carriers) was assessed by using the method from Hixson and Vernier.[44 45]

Ascertainment of healthy ageing

Table 1 shows that healthy ageing was defined, and adapted with minor modifications from the established Cardiovascular Health Study definition,[46] as survival without cardiovascular disease, cancer, lung disease, and severe chronic kidney disease, the absence of cognitive dysfunction, and physical dysfunction (no difficulties with activities of daily living); or as death after age 65 without these conditions. In exploratory analyses, healthy ageing was further disaggregated into disease-free healthy ageing (absence of cardiovascular disease, cancer, lung disease, and severe chronic kidney disease, including deaths related to these conditions), and functional healthy ageing (absence of physical and cognitive dysfunction, and death from dementia; supplementary materials, table A). We also considered an expanded healthy ageing definition adding atrial fibrillation, coronary artery bypass surgery, mild or moderate chronic kidney disease, and diabetes (conditions less likely to be fatal or impact severely on quality of life; an absence of the diseases in the primary definition).

Statistical analysis

We used a Cox proportional hazards model to evaluate the association between time-varying n3-PUFA levels, adjusting for time-varying covariates (updated at each fatty acid measurement), and the likelihood of unhealthy ageing.[47] Time at risk was from the first fatty acid measurement until the first unhealthy ageing event or censoring, including death after age 65 without any defined unhealthy ageing conditions, loss to follow-up, or the latest date of adjudicated follow-up in June 2015. For outcomes that require annual clinical examinations (physical and cognitive function), all participants were assumed to have no incidence between 1999-2005

when clinical examinations were not conducted. The proportional hazards assumption was not violated based on Schoenfeld residuals.[48] α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid levels were assessed individually, and as summed long chain n3-PUFAs (eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid). To incorporate changes over time, we evaluated time-varying n3-PUFA levels as weighted cumulative averages. At each serial measure, we calculated the average of the current and previous measures, with 50% weight assigned to the most recent n3-PUFA measurement and equal weighting for previous n3-PUFA measures. For participants with missing n3-PUFA levels, previous measurements were carried forward (16.0% in 1998, 23.6% in 2005). Fatty acids were evaluated categorically in five quintiles (hereon referred to as groups) as indicator variables, with group cutpoints based on baseline levels. To assess linear trends, groups were assessed as continuous variables after assigning participants the median value in each group. We also evaluated n3-PUFA levels continuously per interquintile range, the difference between the midpoint of the first and fifth quintiles. We explored potential non-linear associations semiparametrically by using restricted cubic splines.

We selected covariates and potential mediators based on biological interest, current or previously observed associations with n3-PUFAs or healthy ageing, and meaningful changes in the exposure risk estimate (±5%). Minimal adjustments included age and sex. Multivariable adjustments additionally included ethnicity, enrolment site, education, income, physical activity, waist circumference, body mass index, alcohol consumption, smoking status, self reported general health status, and family history of myocardial infarction or stroke, or both. Additional diet and circulating fatty acid adjustments included fruit intake, vegetable intake, red meat intake, energy intake, dietary fiber intake, n6-PUFAs, *trans*-16:1, total *trans*-18:1, and total *trans*-18:2 levels. Collinearity among levels of the n3-PUFA biomarkers and other fatty acid biomarkers was not high (Spearman r≤0.45 for all, and r≤0.30 for most). Potential mediators included C-reactive protein, systolic blood pressure, diastolic blood pressure, hypertension drugs, osteoporosis, treated arthritis, and depression. We used the potential mediators to explore what additional associations could exist to these potential pathways. We further adjusted for total fish intake in the model to assess the independent association of n3-PUFAs. We conducted single imputation on covariates with missing data (impute command in Stata), where cases by patterns of missing data were organized in order to conduct efficient missing-value regressions. At each time point, single imputation was performed for missing covariates (0.1% to 6.0% in 1992-93, 1.7% to 15.6% in 1998-99, and 3.0% to 12.5% in 2005-06 for most factors including diet; up to 30% in 2005-06 for anthropometrics, blood measurements and self reported depression) by using up to 29 demographic and risk variables, and up to four additional dietary variables for missing dietary covariates. Previous publications in the Cardiovascular Health Study have confirmed similar results for single versus multiple imputation of covariates.[49] In sensitivity analyses, participants with missing values, and participants with self reported poor health were excluded separately. Interaction by age, sex, n6-PUFAs, and Apolipoprotein E-ε4 genotype for those who had given consent (n=2412) was explored including continuous (linear) multiplicative interaction terms with each n3-PUFA, including the main effects in the model and corrected for multiple comparisons (Bonferroni α=0.003). We additionally evaluated the ratio of n6-PUFA to long chain n3-PUFA, n6-PUFA to n3-PUFA, linoleic acid to α-linolenic acid, and arachidonic acid to eicosapentaenoic acid and docosahexaenoic acid, with the main effects for each ratio in the model. We did not adjust for multiple comparisons (with the exception of our primary outcome and the prespecified hypotheses about each n3-PUFA), but were cautious when interpreting results unrelated to the primary hypothesis; paid close attention to internal consistency and the findings of others; and gave appropriate weight in the interpretation of biological plausibility based on known pathophysiology, biochemistry, and molecular genetics. We used Stata version 14.2 (StataCorp, College Station, TX) for all statistical analyses.[50] We used a two sided α level of 0.05 to assess significance.

Patient and public involvement

No patients were involved in setting the research question or the outcome measures, nor were they involved in developing plans for design or implementation of the study. No patients were asked to advise on interpretation or writing up of results. There are no plans to disseminate the results of the research to study participants or the relevant patient community. The Cardiovascular Health Study results are disseminated to the wider patient community through a study website, hosted at both the Cardiovascular Health Study Coordinating Centerand the National Institutes of Health: National Heart, Lung, and Blood Institute, that highlights notable findings. Such findings are also disseminated to participants on occasion through a study newsletter.

Results

Participant characteristics

Table 2 shows that at baseline, the mean (SD) age was 74.4 (4.8) years, 63.4% of the participants were women, and 10.8% were from non-white groups. Participants with higher long chain n3-PUFAs levels were more likely to be female, white, more educated, have a higher income, and have a healthier lifestyle. Participants in the highest group consumed about one additional weekly serving of fish, compared with the lowest group. Participant characteristics by α-linolenic acid levels were generally similar, except participants

with higher α-linolenic acid levels tended to be high school graduates, with a lower income, consume more alcohol, have a lower body mass index, and have lower C-reactive protein levels (supplementary materials, table F). Levels of n3-PUFAs were similar between healthy agers at baseline versus unhealthy agers excluded at baseline (supplementary materials, table G). Characteristics of participants who were deceased before baseline, with no blood fatty acid measurements, and who consumed fish oil were largely similar to those included in the analysis, except those who were deceased at baseline were more likely to be older, male, white, less educated, have a lower income, and report fair or poor health status (supplementary materials, table H). Across 13 years of serial measures, the mean cohort values of each n3-PUFA increased slightly over time, although not meaningfully so (supplementary materials, fig A). Spearman correlations for serial levels of each n3-PUFA, reflecting reproducibility over time, ranged from 0.40 to 0.65 for α-linolenic acid, and 0.59 to 0.80 for eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid (supplementary materials, table I). Pairwise correlations between different long chain n3-PUFAs were generally low to modest (r=0.07 to 0.57), and lower with α-linolenic acid (r<0.01 to 0.25) (supplementary materials, table I).

Groups of n3-PUFA and healthy ageing

During 21 803 person years of follow-up, 2330 (89%) participants experienced unhealthy ageing, with 292 (11%) showing healthy ageing (positive deviance). Among those censored, only four participants died prematurely aged under 70 owing to a cause not included in our definition. Figure 1 shows that after multivariable adjustment for demographic, lifestyle, cardiovascular risks, dietary habits, and other phospholipid fatty acids, higher long chain n3-PUFA (but not α-linolenic acid) levels were associated with a lower likelihood of unhealthy ageing. Overall, participants in the highest group of total long chain n3-PUFAs had an 18% (95% confidence interval 3% to 30%; P=0.001) lower risk of unhealthy ageing. When individual n3-PUFAs were evaluated separately, participants in the highest eicosapentaenoic acid or docosapentaenoic acid groups (but not α-linolenic acid or docosahexaenoic acid) had a 24% (11% to 35%; P<0.001) and 18% (6% to 29%; P=0.003) lower risk of unhealthy ageing respectively, compared with the lowest group. Findings were not appreciably altered after adjustment for potential mediators (not shown).

Continuous linear assessment

Figure 2 shows that in linear models, higher levels of long chain n3-PUFAs, but not α-linolenic acid, were consistently associated with a lower likelihood of unhealthy ageing. For each interquintile range, the risk of unhealthy ageing was lower by 15% (95% confidence interval 6% to 23%) for eicosapentaenoic acid, 16% (5% to 24%) for docosapentaenoic acid, and 18% (7% to 28%) for total long chain n3-PUFA. With further adjustments for potential mediators, docosahexaenoic acid was also associated with a 12% (0% to 23%) lower risk of unhealthy ageing, whereas results for the remaining long chain n3-PUFAs were not appreciably altered.

Figure 3 shows that when we evaluated potential non-linear relations with healthy ageing by using restricted cubic splines, none of the associations revealed a statistically significant departure from linearity. The top and bottom 1% of participants were omitted as outliers to provide better visualization. Evidence for non-linearity (P_{curve}) was calculated by performing a likelihood ratio test between a multivariable model with all spline terms versus a multivariate model with only the linear term, while evidence for linearity was calculated by performing a likelihood ratio test between a multivariable model without spline terms versus a multivariable model with only the linear term. No evidence for non-linearity was found, where P_{curve}=0.57, P_{curve}=0.25, P_{curve}=0.10, and P_{curve}=0.48 for α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, respectively, despite visually suggesting a possible threshold effect.

Sensitivity analyses

In sensitivity analyses, results remained largely similar after excluding participants with self reported poor health (supplementary materials, figs B and C). For example, excluding participants with missing values for docosapentaenoic acid, the hazard ratio for exclusion was 0.85 (95 confidence interval 0.75 to 0.98) versus a hazard ratio of 0.84 (0.75 to 0.94) using imputation. We found similar results when we used the expanded healthy ageing definition incorporating atrial fibrillation, coronary artery bypass surgery, chronic kidney disease and diabetes (supplementary materials, fig D). When total fish intake was added to the multivariable model, docosahexaenoic acid was inversely associated with unhealthy aging by 17% (4% to 27%), whereas results for the remaining n3-PUFAs were not appreciably altered (data not shown).

Exploratory analyses

When healthy ageing was disaggregated into disease-free versus functional healthy ageing, higher α-linolenic acid levels were inversely associated with non-disease-freehealthy ageing, with 19% lower risk (95% confidence interval 9% to 29%) per interquintile range (supplementary materials, fig D), but was not noticeably associated with functional healthy ageing. Eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, individually and in combination, were generally inversely associated with both outcomes, with findings relatively stronger for functional versus disease-free healthy ageing (supplementary materials, fig D).

The association between n3-PUFA levels and healthy ageing did not vary by age, sex, n6-PUFA levels, or Apolipoprotein E genotype after Bonferroni

Table 2 | Baseline participant characteristics by quintile groups of summed eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid from the Cardiovascular Health Study in 1992-93 (n=2369). Values are numbers (percentages) unless stated otherwise

	Group				
Characteristic	**1 (n=470)**	**2 (n=480)**	**3 (n=477)**	**4 (n=476)**	**5 (n=466)**
Demographics					
Mean (SD) age (years)	74.5 (5.1)	74.3 (4.7)	74.4 (4.7)	74.1 (4.8)	74.5 (4.9)
Female	288 (61.3)	290 (60.4)	311 (65.2)	304 (63.9)	310 (66.5)
Race or ethnicity:					
White	450 (95.7)	451 (94.0)	421 (88.3)	407 (85.5)	384 (82.4)
Non-white groups	20 (4.3)	29 (6.0)	56 (11.7)	69 (14.5)	82 (17.6)
Education:					
<High school	134 (28.5)	115 (24.0)	124 (26.0)	84 (17.6)	65 (13.9)
High school	143 (30.4)	149 (31.0)	148 (31.0)	138 (29.0)	124 (26.6)
Some college	108 (23.0)	121 (25.2)	118 (24.7)	113 (23.7)	104 (22.3)
College graduate	85 (18.1)	95 (19.8)	87 (18.2)	141 (29.6)	173 (37.1)
Annual income:					
<$12 000	111 (23.6)	94 (19.6)	97 (20.3)	95 (20.0)	76 (16.3)
$12 000-$24 999	192 (40.9)	186 (38.8)	171 (35.8)	135 (28.4)	118 (25.3)
$25 000-$49 999	123 (26.2)	141 (29.4)	155 (32.5)	162 (34.0)	135 (29.0)
>$50 000	44 (9.4)	59 (12.3)	54 (11.3)	84 (17.6)	137 (29.4)
Enrolment site:					
Bowman Gray, NC	138 (29.4)	142 (29.6)	133 (27.9)	110 (23.1)	76 (16.3)
Davis, CA	129 (27.4)	130 (27.1)	128 (26.8)	121 (25.4)	95 (20.4)
Hopkins, MD	126 (26.8)	121 (25.2)	124 (26.0)	120 (25.2)	52 (11.2)
Pittsburgh, PA	77 (16.4)	87 (18.1)	92 (19.3)	125 (26.3)	243 (52.1)
Lifestyle					
Smoking status:					
Never smoker	233 (49.6)	234 (48.8)	255 (53.5)	244 (51.3)	211 (45.3)
Former smoker	173 (36.8)	202 (42.1)	180 (37.7)	194 (40.8)	221 (47.4)
Current smoker	64 (13.6)	44 (9.2)	42 (8.8)	38 (8.0)	34 (7.3)
Mean (SD) physical activity (mcal/week)	1.3 (1.5)	1.3 (1.4)	1.1 (1.3)	1.2 (1.4)	1.2 (1.4)
Mean (SD) alcohol (servings/week)	2.5 (6.0)	2.2 (5.5)	2.6 (6.7)	3.1 (7.1)	2.8 (6.1)
Mean (SD) body mass index (kg/m^2)	26.3 (4.4)	26.9 (4.6)	26.7 (4.3)	26.6 (4.5)	26.2 (4.4)
Mean (SD) waist circumference (cm)	96.4 (12.6)	97.2 (13.1)	97.2 (13.2)	96.8 (13.0)	94.5 (12.8)
Mean (SD) blood pressure (mm Hg):					
Systolic	137 (20.6)	136 (20.7)	137 (21.3)	135 (20.1)	133 (21.5)
Diastolic	72 (9.8)	72 (10.2)	71 (11.9)	71 (11.3)	71 (10.5)
Medical history					
Health status (self report):					
Excellent or very good	209 (44.5)	246 (51.3)	219 (45.9)	248 (52.1)	233 (50.0)
Good	184 (39.1)	160 (33.3)	192 (40.3)	165 (34.7)	171 (36.7)
Fair or poor	77 (16.4)	74 (15.4)	66 (13.8)	63 (13.2)	62 (13.3)
Family history of myocardial infarction or stroke	136 (28.9)	155 (32.3)	146 (30.6)	143 (30.0)	125 (26.8)
Hypertension drugs	163 (34.7)	193 (40.2)	192 (40.3)	192 (40.3)	178 (38.2)
Lipid lowering drugs	18 (3.8)	26 (5.4)	17 (3.6)	26 (5.5)	20 (4.3)
Prevalent diabetes	93 (19.8)	90 (18.8)	108 (22.6)	95 (20.0)	68 (14.6)
Have osteoporosis	30 (6.4)	43 (9.0)	41 (8.6)	35 (7.4)	45 (9.7)
Treated arthritis	112 (23.8)	119 (24.8)	113 (23.7)	112 (23.5)	116 (24.9)
Mean (SD) depression score*	3.9 (3.9)	4.0 (4.1)	4.3 (4.2)	4.3 (4.4)	4.1 (4.1)
Blood measurements					
Mean (SD) C-reactive protein (nmol/L)	31.4 (79.1)	28.6 (50.5)	29.5 (53.3)	23.8 (30.5)	26.7 (41.0)
Mean (SD) low density lipoprotein (mmol/L)	3.30 (0.92)	3.24 (0.82)	3.41 (0.79)	3.28 (0.87)	3.27 (0.77)
Mean (SD) high density lipoprotein (mmol/L)	1.40 (0.38)	1.37 (0.36)	1.38 (0.35)	1.41 (0.36)	1.45 (0.39)
Mean (SD) triglycerides (mmol/L)	1.63 (1.08)	1.66 (0.95)	1.66 (0.86)	1.57 (0.93)	1.46 (0.79)
Median (range) total fatty acid†:					
ω-6	35.0 (28.9-38.5)	34.8 (28.8-38.2)	34.5 (28.6-38.0)	34.0 (27.5-37.3)	32.8 (23.5-36.4)
trans-16:1	0.26 (0.10-0.48)	0.25 (0.13-0.43)	0.25 (0.08-0.41)	0.25 (0.10-0.42)	0.25 (0.06-0.46)
trans-18:1	2.26 (0.58-8.46)	2.03 (0.60-5.25)	1.88 (0.30-4.15)	1.83 (0.54-4.15)	1.65 (0.23-4.27)
trans-18:2	0.23 (0.10-1.07)	0.24 (0.10-0.78)	0.25 (0.10-0.69)	0.25 (0.12-0.92)	0.25 (0.09-0.58)
Diet history‡					
Mean (SD) energy intake (kcal/d)	2019 (621)	1957 (558)	1988 (633)	2000 (561)	1931 (586)
Mean (SD) food intake (servings/d):					
Fruit	2.1 (1.0)	2.1 (1.0)	2.2 (1.1)	2.4 (1.0)	2.4 (1.0)
Vegetables	2.8 (1.5)	2.9 (1.5)	3.0 (1.4)	3.2 (1.5)	3.2 (1.3)
Non-processed red meat	0.5 (0.3)	0.5 (0.3)	0.5 (0.3)	0.4 (0.3)	0.4 (0.3)
Processed meat	0.4 (0.4)	0.4 (0.3)	0.4 (0.3)	0.3 (0.3)	0.3 (0.3)
Mean (SD) total fish intake (servings/week)	1.3 (1.2)	1.4 (1.2)	1.6 (1.2)	1.9 (1.4)	2.3 (1.6)
Mean (SD) wholegrain intake (g/d)	32.8 (20.9)	32.5 (18.6)	33.2 (20.4)	34.8 (19.6)	35.0 (21.5)
Mean (SD) dietary fibre (g/d)	26.8 (10.2)	26.6 (8.9)	27.3 (9.9)	28.4 (10.1)	27.8 (9.7)

*Depression is self reported using the 10 item Centers for Epidemiological Studies' Depression Scale.[38]
†Expressed as percentage of total fatty acids.
‡Values are the mean of food frequency questionnaires completed in 1989-90 and 1995-96.

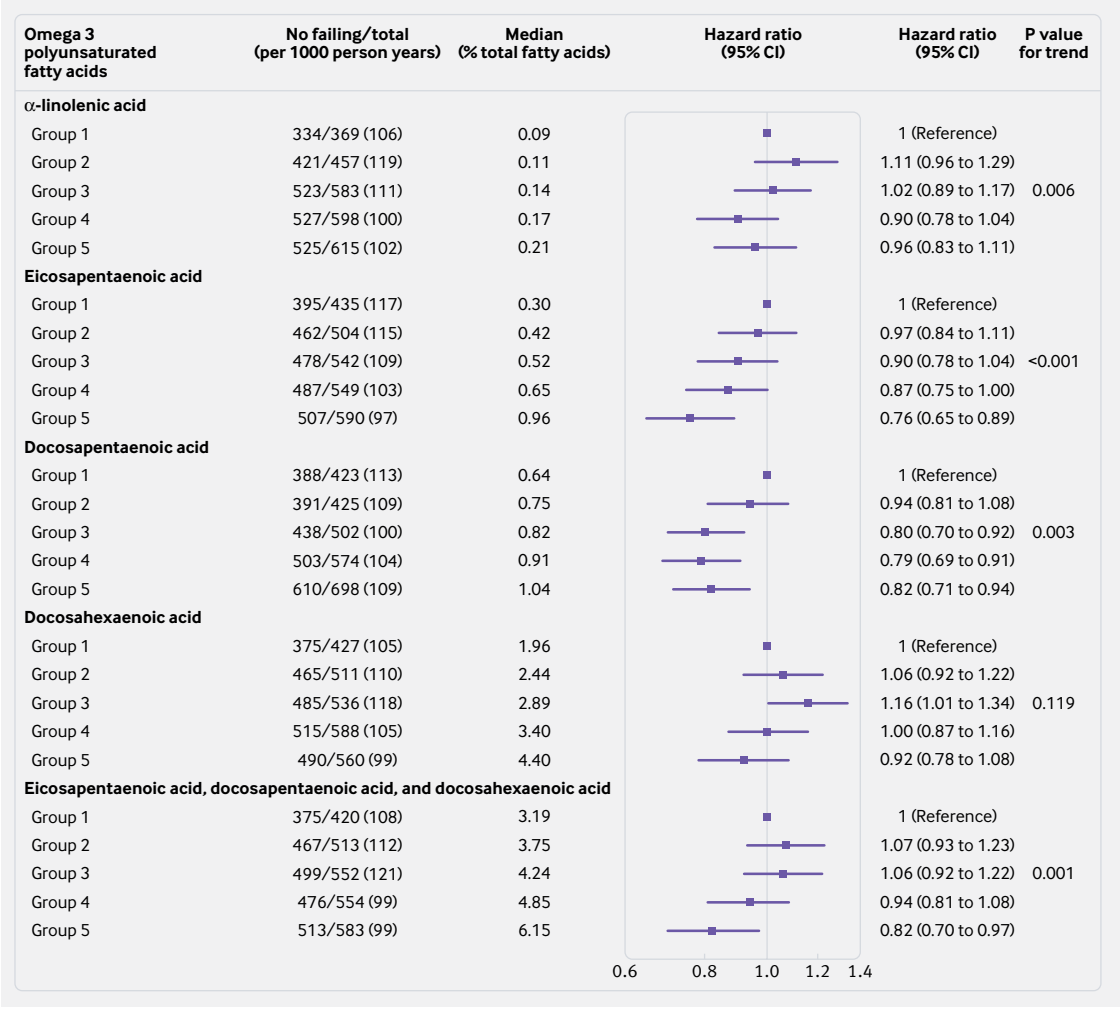

Fig 1 | Omega 3 polyunsaturated fatty acids and the risk of unhealthy ageing in the Cardiovascular Health Study after 22 years of maximum follow-up among 2622 older adults

correction (supplementary materials, table J). Risk estimates in women for α-linolenic acid and docosapentaenoic acid were slightly more inverse compared with men, but results were not statistically significant (P=0.24 and P=0.10, respectively). The only exception was a potential interaction between age and α-linolenic acid (P=0.003 for interaction): α-linolenic acid levels were borderline associated with a lower risk of unhealthy ageing among those below the median age (74 years) at baseline (hazard ratio 0.87 per interquintile range, 95% confidence interval 0.75 to 1.01), but not those above the median age (0.99, 0.84 to 1.17). None of the n6-PUFA to n3-PUFA ratios were associated with healthy ageing. For example, per unit of the n6-PUFA to long chain n3-PUFA ratio, the hazard ratio was 1.00 (95% confidence interval 0.96 to 1.05; P=0.85). Hazard ratios for the other ratios were: n6-PUFA to total n3-PUFA, 1.00 (0.95 to 1.05; P=0.86); linolenic acid to α-linolenic acid, 1.00 (1.00 to 1.00; P=0.81); arachidonic acid to eicosapentaenoic acid and docosahexaenoic acid, 1.01 (0.92 to 1.11; P=0.86).

Discussion

In this prospective cohort study among community based older US adults, higher serial levels of eicosapentaenoic acid, docosapentaenoic acid, and summed long chain n3-PUFAs, but not α-linolenic acid biomarkers, were associated with a higher likelihood of healthy ageing. In general, about 18% to 24% difference in risk was observed across the five groups. Docosahexaenoic acid was not noticeably associated with healthy ageing, although the risk estimate tended toward benefit, and was statistically significant after adjusting for total fish intake. Associations were generally linear and were robust to various sensitivity analyses. This is the first investigation on serial n3-PUFA biomarkers and healthy ageing.

Mechanisms and implications

Long chain n3-PUFAs show several physiological effects that support the biologic plausibility of our results.[7 51] Human trials show favorable effects on blood pressure, endothelial function, plasma triglycerides, heart rate, and potentially inflammation.[7]

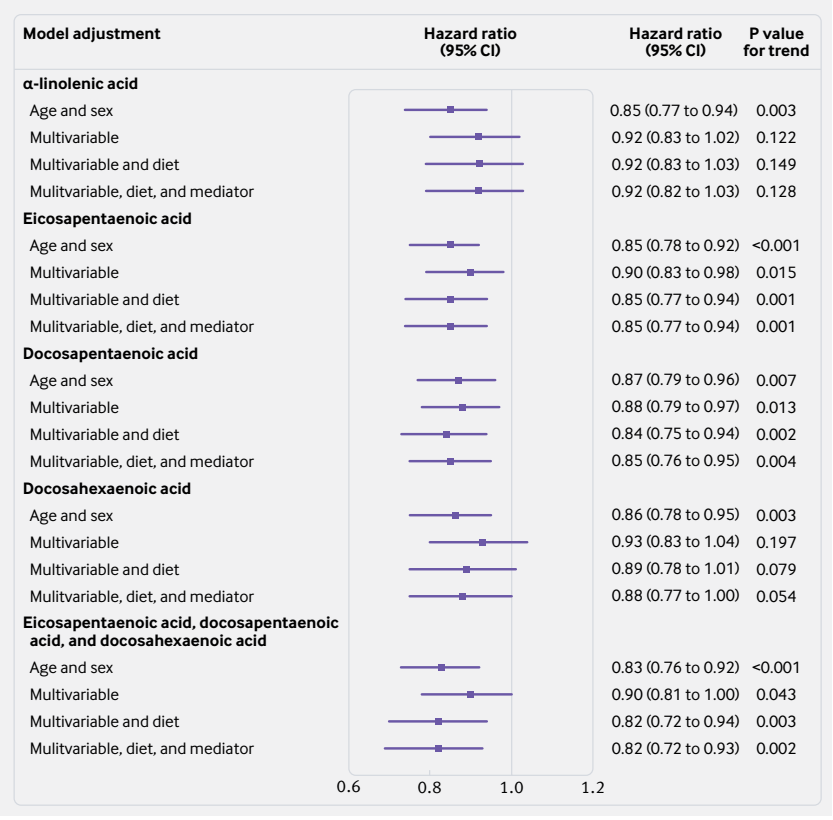

Fig 2 | Hazard ratios (95% confidence intervals) of 2330 unhealthy ageing events per interquintile range (IQR) of omega 3 polyunsaturated fatty acids (n3-PUFAs) after 22 years of maximum follow-up among 2622 older adults. The IQR is equivalent to the difference between the midpoint of the 1st and 5th quintiles, estimated to be 0.12%, 0.64%, 0.40%, 2.40% and 2.90% for the n3-PUFAs, respectively

Experimental and animal studies suggest potential benefits for arrhythmias,[7 51] carcinogenesis,[8] bone mass,[52] and neurogenesis.[53] Evidence for other effects, such as those on cognitive function and mental health, is mixed.[54-56] Mechanistically, long chain n3-PUFA alter fluidity of cellular membranes and responses of transmembrane protein receptors, influence gene expression by binding to fatty acid specific receptors, and serve as precursors to bioactive eicosanoids and specialized resolvers of inflammation.[7-9 53]

Omega 3 levels can be influenced by both diet and metabolism. Metabolic effects are confirmed by genetic studies. For example, common genetic variation in the *FADS1/2* and *ELOVL2* genes explain between 0.4 to 9 of the variance in n3-PUFA levels.[57] Additionally, docosahexaenoic acid was associated with lower risk after adjustment for total fish intake. This could reflect improved precision (statistical power) after adjustment for an important covariate or it could suggest a stronger role of metabolically, rather than dietary, determined levels of docosahexaenoic acid. Diet also plays a clear role in n3-PUFA levels. In the Cardiovascular Health Study, the correlation between self reported estimated dietary intake of eicosapentaenoic acid and docosahexaenoic acid and plasma phospholipid n3-PUFA biomarkers is ~0.45.[30] As each of these measures includes (uncorrelated) errors in comparison to the true habitual consumption of eicosapentaenoic acid and docosahexaenoic acid, the correlation between the n3-PUFA biomarkers and true habitual consumption is higher, likely in the range of ~0.7.[58] Based on observed differences in fish intake in this cohort, the difference in long chain n3-PUFA levels across the five groups in our investigation corresponds to a dietary difference of about one fish serving/week.

The generally similar findings for total and individual long chain n3-PUFAs suggest a potential class effect. Long chain n3-PUFA levels are only modestly intercorrelated, suggesting that shared and complementary pathways and molecular mechanisms are possible.[59] Although docosahexaenoic acid was not associated with healthy ageing in the primary analyses, its risk estimate was similar to that for eicosapentaenoic acid (0.89 *v* 0.85, respectively), and the confidence intervals of each estimate also overlapped. Additionally, docosahexaenoic acid was associated with healthy ageing after adjustment for total fish intake. Thus, although our findings cannot confirm an association for docosahexaenoic acid alone, they also do not exclude the possibility of benefit. Eicosapentaenoic acid and docosahexaenoic acid levels are each more strongly determined by diet,[43] whereas docosapentaenoic acid is more strongly determined by endogenous metabolism.[7] The similar associations of eicosapentaenoic acid and docosapentaenoic acid reported here suggest that achieved circulating levels of long-term n3-PUFAs could be most relevant for health effects.

Contrary to concerns about clinically meaningful competition between n3-PUFAs and n6-PUFAs, we found no evidence for any effects of various n6-PUFA ton3-PUFA ratios. Although power was insufficient to confirm any statistical interaction between long chain n3-PUFAs and Apolipoprotein E genotypes, the consistent observed differences in the central risk estimates are interesting. These new findings highlight the need for further investigation of the potential interaction between Apolipoprotein E genotypes, long chain n3-PUFAs, and healthy ageing.

We found little evidence for associations of α-linolenic acid levels with healthy ageing. Although α-linolenic acid may share some functional and physiological attributes with long chain n3-PUFAs,[51] α-linolenic acid appears to be less biologically active, and with limited in vivo conversion to eicosapentaenoic acid (between 0.2% to 8%) and subsequently docosahexaenoic acid (between 0% to 4%).[7 60] Plasma phospholipid α-linolenic acid may also not reflect dietary intake well, given its rapid oxidation.[13 61] In exploratory analyses, an association was seen between α-linolenic acid and disease-free (as opposed to functional) healthy ageing, as well as an interaction with age. These findings require confirmation in further investigations and should be interpreted with caution. Although women convert α-linolenic acid to eicosapentaenoic acid and long chain n3-PUFAs at a higher rate than men, this conversion is still quite low.[60] We did not find evidence for interaction of α-linolenic acid and healthy ageing by sex.

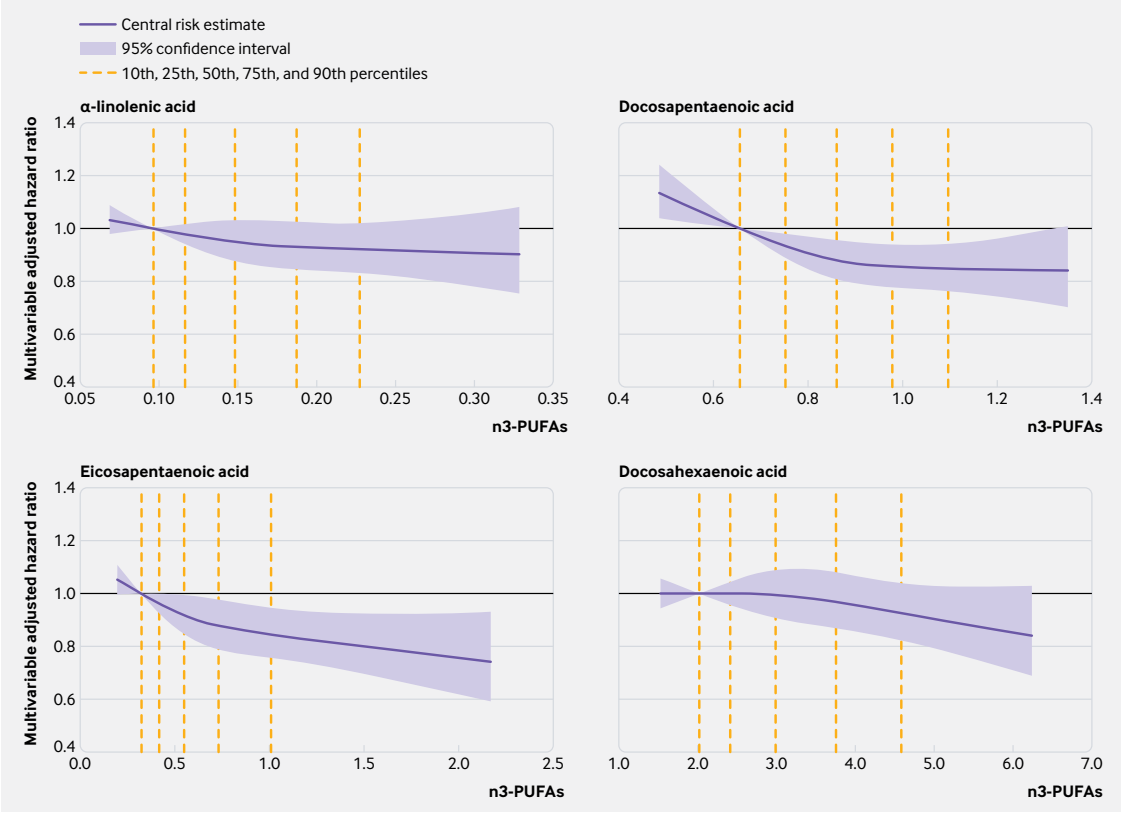

Fig 3 | Multivariable-adjusted relationship of plasma phospholipid omega 3 polyunsaturated fatty acid (n3-PUFA) levels with risk of unsuccessful healthy ageing, evaluated using restricted cubic splines

Comparison with other studies

No previous study has reported on associations of n3-PUFA biomarkers and healthy ageing. A few studies have examined associations between baseline measures of n3-PUFA and certain individual components of healthy ageing. For example, higher circulating long chain n3-PUFAs have been inversely associated with fatal coronary heart disease,[12] breast cancer,[17] and cognitive function (including dementia, Alzheimer's disease, and brain ageing),[62-64] yet also positively associated with prostate cancer and with inconclusive evidence for physical function.[16 65] Consistent with our results, circulating α-linolenic acid levels have more limited associations with components of healthy ageing in previous studies, including modest inverse associations with fatal coronary heart disease,[12] no significant associations with breast and prostate cancer,[16 17] and limited evidence for cognitive and physical function. Our findings build on and expand these previous results by evaluating serial measurements of biomarkers in relation to healthy ageing.

Strengths and limitations

The current study has several strengths. This longitudinal cohort of older US adults was followed for nearly 25 years, providing time for the development of a large number of ageing events, and adequate statistical power. The community based design improves generalizability, and regular physical examinations ensured that demographics and other risk factors were well measured, which may help to minimize confounding. Unlike investigations into longevity alone or incidence of specific diseases, this investigation of healthy ageing considers the quality of health later in life, living to a reasonable age without major disabling diseases, and not dying of common conditions related to lifestyle. Our definition of healthy ageing incorporated well measured clinical events and objective tests of physical and cognitive function. Repeated n3-PUFA biomarker measurements over 13 years helped to capture changes in and cumulative effects of n3-PUFA levels over time, and allowed for the evaluation of individual n3-PUFAs. Biomarkers incorporate influences of both diet and metabolism, and compared with self reported dietary estimates, are not influenced by reporting bias or inaccuracies in food composition databases. Long term correlations of levels of n3-PUFAs over time indicate that levels at one time point reasonably predict levels many years later, even with changes in diet and metabolism.

Potential limitations should be considered. Although healthy ageing has a common conceptual framework, its operational definition has varied across studies (eg, based on available outcome measures and the research question of interest).[5 66] We focused on functional and disease-free healthy ageing and did not include psychosocial factors or other subjective measures of satisfaction in life. We also did not explore the associations with multiple individual components of

healthy ageing as that is currently beyond the scope and focus of the present paper, although findings highlight the need for further investigation in future studies. We had robust disease classification methods, but some degree of misclassification is still likely, which could most often attenuate findings toward the null. For example, associations were partly attenuated when atrial fibrillation, chronic kidney disease, and diabetes were included in sensitivity analysis, the assessment of which partly relied on administrative databases which could over or under ascertain cases.[46] Imputation of missing covariates will lead to some degree of imprecision or bias. However, results excluding missing values were similar, and the proportion of missing covariates was small for most factors. Even using serial biomarkers, residual measurement error may be present in n3-PUFA exposures; the prospective design makes it more likely that such errors would be random with respect to events occurring many years later, causing underestimation of true associations. Although the population of older adults is highly relevant to healthy ageing, results may not necessarily be generalizable to younger populations.

The possibility of residual confounding by imprecisely measured or unknown factors also cannot be excluded for an observational study. Generally, adjustment for education and n6-PUFA levels influenced estimates most: upward (less protective) with adjustment for education, and downward (more protective) with adjustment for n6-PUFA. Both covariates were reasonably well measured and also had opposing directions of confounding. A strength of this investigation was the availability of a wide range of standardized measures for covariate adjustment to consider unmeasured potential confounders. Thus, we evaluated models adjusted for age and sex; further adjusted for race or ethnicity, enrolment site, education, income, physical activity, waist circumference, body mass index, alcohol consumption, smoking status, self reported general health status, and family history of myocardial infarction, or stroke, or both; further adjusted for fruit intake, vegetable intake, energy intake, dietary fiber, non-processed red meat intake, plasma phospholipid n6-PUFAs, total *trans*-16:1, total *trans*-18:1, and *trans*-18:2; further adjusted for total fish intake; and further adjusted for potential mediators such as C-reactive protein, systolic blood pressure, diastolic blood pressure, hypertension drugs, osteoporosis, treated arthritis, and depression. Any unmeasured confounders would have to be strongly associated with both the exposure and the outcome, conditional on all the variables already in the model. Thus, it seems unlikely that either poorly measured or unmeasured confounders could fully account for our findings. For docosahexaenoic acid, adjustment for total fish intake led to a more protective risk estimate and greater precision. Self reported fish intake may not be measured with high accuracy, so residual confounding in this covariate could underestimate the true association of docosahexaenoic acid with healthy ageing.

Conclusions

Among older adults, a higher cumulative level of the circulating long chain n3-PUFAs eicosapentaenoic acid, docosahexaenoic acid, and eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid combined were associated with a lower likelihood of unhealthy ageing. Docosahexaenoic acid from seafood and α-linolenic acid from plants were not associated with a lower likelihood of unhealthy ageing. These findings encourage the need for further investigations into plausible biological mechanisms and interventions related to n3-PUFAs for the maintenance of healthy ageing, and to support guidelines for increased dietary consumption of fish in older adults.[67 68]

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Contributors: HTML, MCOO, RNL, BM, PHMC, MCO, ABN, DSS, and DM developed the study concept and design. HTML, MCOO, RNL, BM, XS, IBK, DSS, and DM contributed to the acquisition, analysis, and interpretation of data. HL conducted the statistical analysis. HL and DM wrote the first draft of the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content. DM, DSS, RNL and BM obtained the funding for the study data and analysis. HTML, MCOO, RNL, DSS, and DM provided administrative, technical and material support for the study analysis. DM is the study supervisor. All authors had full access to the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis. HTML is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Ethical approval: All protocols were approved at the institutional review board of each participating university.

Data sharing: No additional data are available.

Transparency: The manuscript's guarantor affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

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Supplementary materials: Supplementary tables A-J and figures A-D