Omega-3 and omega-6 polyunsaturated fatty acid biomarkers and sleep: a pooled analysis of cohort studies

On behalf of the Fatty Acids and Outcomes Research Consortium (FORCE)

Authors

Rachel A Murphy, Nathan Tintle, William S Harris, Maryam Darvishian, Matti Marklund, Jyrki K Virtanen, Sari Hantunen, Vanessa D de Mello, Jaakko Tuomilehto, Jaana Lindström, Matthew A Bolt, Ingeborg A Brouwer, Alexis C Wood, Mackenzie Senn, Susan Redline, Michael Y. Tsai, Vilmundur Gudnason, Gudny Eiriksdottir, Eva Lindberg, Aladdin H Shadyab, Buyun Liu, Mercedes Carnethon, Matti Uusitupa, Luc Djousse, Ulf Risérus, Lars Lind, Rob M van Dam, Woon-Puay Koh, Peilin Shi, David Siscovick, Rozenn N Lemaitre, Dariush Mozaffarian

Author Affiliations

Cancer Control Research, BC Cancer, 675 W 10th Ave, Vancouver, BC, Canada (RAM, MD)

School of Population & Public Health, Faculty of Medicine, The University of British Columbia, 2206 East Mall, Vancouver, BC V6T 1Z3, Canada (RAM)

Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Yliopistonranta 1, 70210 Kuopio, Finland (JKV, SH, VDdeM, MU)

Finland Institute for Health and Welfare, Helsinki, Finland (JL)

Public Health, University of Helsinki, Finland (JT)

National Institute for Health and Welfare, Helsinki, Finland (JT)

National School of Public Health, Madrid, Spain (JT)

Department of Mathematics and Statistics, Dordt College, 700 7th St NE, Sioux Center, IA
51250, United States (NT, MAB)
Department of Health Sciences, Faculty of Science, Vrije Universiteit Amsterdam, and
Amsterdam Public Health Research Institute, De Boelelaan 1105, 1081 HV Amsterdam,
Netherlands (IAB)
USDA/ARS Children’s Nutrition Research Center, Department of Pediatrics, Baylor College of
Medicine, 1 Baylor Plaza, Houston, TX 77030, United States (ACF, MS)
Department of Medicine, Brigham and Women's Hospital, Boston, MA, United States (LD)
Division of Sleep Medicine, Harvard Medical School, Boston, MA, United States (SR)
Division of Pulmonary, Critical Care, and Sleep Medicine, Beth Israel Deaconess Medical
Center, Boston, MA, United States (SR)
Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN,
United States (MYT)
The George Institute for Global Health, Faculty of Medicine, University of New South Wales,
Sydney, Australia (MM)
Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore,
MD, USA (MM)
Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala,
Sweden (MM, UR, LL)
Icelandic Heart Association Research Institute, Holtasmári 1, Kópavogur, Iceland, Iceland (VG, GE)
Department of Internal Medicine, Sanford School of Medicine, University of South Dakota,
1400 W 22nd St, Sioux Falls, SD 57105, United States (WSH)
Fatty Acid Research Institute, Sioux Falls, SD 57106, United States (WSH, NT)
Department of Medical Sciences, Respiratory, Allergy and Sleep Research, Uppsala University, Sweden (EL)

Herbert Wertheim School of Public Health and Human Longevity Science, University of California, San Diego, La Jolla, CA, United States (AHS)

Saw Swee Hock School of Public Health, National University of Singapore and National University Health System, Republic of Singapore (RMvD)

Healthy Longevity Translational Research Programme, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117545, Singapore (WPK)

Singapore Institute for Clinical Sciences, Agency for Science Technology and Research (A*STAR) Singapore 117609, Singapore (WPK)

Department of Epidemiology, College of Public Health, University of Iowa, Iowa City, IA, United States (BL)

Department of Preventive Medicine, Northwestern University, Chicago, IL, United States (MC)

New York Academy of Medicine, United States (DS)

Department of Medicine, Cardiovascular Health Research Unit, University of Washington, Seattle WA, United States (RNL)

Friedman School of Nutrition Science and Policy, Tufts University, 150 Harrison Ave, Boston, MA 02111, United States (DM, PS, MM)

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**Corresponding Author:** Rachel Murphy, 167-2206 East Mall, University of British Columbia, Vancouver, BC, V6T 1Z3 Canada. Telephone: 604-822-1397, Email: Rachel.murphy@ubc.ca

**Running Title:** Blood polyunsaturated fatty acids and sleep
Abbreviations: AA; Arachidonic acid, ALA; alpha-linolenic acid, AGES-R: Age, Gene/Environment Susceptibility Study-Reykjavik; CASS, Chicago Area Sleep Study; CHS, Cardiovascular Health Study, CI; Confidence interval, DHA; docosahexaenoic acid, DPA; docosapentaenoic acid, DPS, Finnish Diabetes Prevention Study, EPA; eicosapentaenoic acid, FHS, Framingham Heart Study; KIHD, Kuopio Ischaemic Heart Disease Risk Factor Study; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; POEM, Prospective Investigation of Obesity, Energy and Metabolism; PUFA; polyunsaturated fatty acids, SCHS, Singapore Chinese Health Study; TG; triglycerides, ULSAM-50, Uppsala Longitudinal Study of Adult Men-50; ULSAM-70, Uppsala Longitudinal Study of Adult Men-70
ABSTRACT

Background: n-3 and n-6 polyunsaturated fatty acids (PUFAs) have physiologic roles in sleep processes, but little is known regarding circulating n-3 and n-6 PUFA and sleep parameters.

Objective: To assess associations between biomarkers of n-3 and n-6 PUFA intake with self-reported sleep duration and difficulty falling sleeping in the Fatty Acids and Outcome Research Consortium.

Methods: Harmonized, de novo, individual-level analyses were performed and pooled across 12 cohorts. Participants were between 35 to 96 years old and from 5 nations. Circulating measures included alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), EPA+DPA+DHA, linoleic acid and arachidonic acid. Sleep duration (10 cohorts, N=18,791) was categorized as short (≤6 hours), 7-8 hours (reference) or long (9+ hours). Difficulty falling sleeping (8 cohorts, N=12,500) was categorized as yes or no. Associations between PUFAs, sleep duration, and difficulty falling sleeping were assessed by cross-sectional multinomial logistic regression using standardized protocols and covariates. Cohort-specific multivariable-adjusted odds ratios (ORs) per quintile of PUFAs were pooled with inverse-variance weighted meta-analysis.

Results: In pooled analysis adjusted for sociodemographics and health status, participants with higher very long-chain n-3 PUFAs were less likely to have long sleep duration. Comparing top vs. bottom quintiles, the multivariable-adjusted OR (95% confidence interval, CI) for long-sleep was 0.78 (0.65, 0.95) for DHA and for EPA+DPA+DHA, 0.76 (0.63, 0.93). Significant associations were not identified for ALA and n-6 PUFA with short sleep duration, or difficulty falling sleeping.
Conclusions: Participants with higher levels of very long-chain n-3 PUFAs were less likely to have long sleep duration. While objective biomarkers reduce recall bias and misclassification, the cross-sectional design limits assessment of the temporal nature of this relationship. These novel findings across 12 cohorts highlight the need for experimental and biological assessments of very long-chain n-3 PUFAs and sleep duration.

Keywords: sleep quality, omega-3, fatty acids, diet, public health, biomarkers

INTRODUCTION

A number of epidemiologic studies show that short sleep (≤6 hours/day) is associated with a variety of physical impairments (1), increased risk of all-cause mortality (2–5), cardiovascular disease (6,7), and incident diabetes (8,9). In addition, similar and even stronger chronic disease and mortality risk relationships have been observed among people who report long sleep duration (9+ hours/day) (2,3,5,10). Independent of duration, as a parameter of sleep quality, difficulty sleeping has been linked to angina (11) and is a feature of insomnia that is associated with increased risk of cardiovascular disease and mortality (12,13).

The American Academy of Sleep Medicine recommends that adults aged 18-60 years sleep 7 or more hours per night (14). The National Sleep Foundation has a similar lower recommendation but places an upper limit of 8 hours for people age 65 and older and 9 hours for people aged 18-64 (15). According to national data from the United States, 35% of adults report insufficient sleep (≤6 hours) (16), and sleep deprivation has been described as a major public health problem by the Centers for Disease Control and Prevention (17).
Certain nutrients may have physiologic effects on sleep regulation, particularly n-3 and n-6 polyunsaturated fatty acids (PUFAs). Docosahexaenoic acid (DHA, 22:6n-3) is important for sleep regulation (18) through its role in regulating melatonin production (19). A study of 63 obese adults with sleep apnea found that higher tissue levels of DHA were associated with better sleep (20) and lower risk of severe apnea (21). In a randomized trial among 362 children, those with lower blood levels of DHA and lower DHA: arachidonic acid (AA, 20:4n-6) ratios at baseline had more sleep disturbances. DHA supplementation for 16 weeks resulted in longer sleep (=58 min) and fewer wakings (-7/night) in a subset of 42 children with sleep actigraphy measures (22). Larger cohort studies are very limited. Among 405 Mexican adolescents, higher vs. lower plasma DHA (across quartiles) was associated with 32 minutes more sleep (23). Oily fish intake was positively associated with sleep quality among 677 adults in Ecuador (24). n-6 PUFA may also influence sleep. AA is a metabolically regulated precursor of a prostaglandin D2, a potent sleep promoter (25), suggesting a possible role of n-6 PUFAs with sleep.

Very few studies of sleep metrics have assessed blood biomarkers of PUFA intake, which provide objective measures of dietary intake and assessment of individual PUFA including plant-derived alpha-linolenic acid (18:3n-3, ALA); seafood-derived, very long-chain eicosapentaenoic acid (20:5n-3, EPA), docosapentaenoic acid (22:5n-3, DPA), and DHA; plant oil derived linolenic acid (18:2n-6, LA); and metabolically regulated AA. We conducted harmonized, de novo, individual-level analyses within 12 studies in the Fatty Acids and Outcomes Research Consortium (FORCE) to assess relationships between n-3 and n-6 PUFA biomarkers and sleep. We hypothesized that lower levels of both PUFA families would be associated with suboptimal sleep including greater risk of short and long sleep, and difficulty falling sleeping.
SUBJECTS and METHODS

Cohorts and study variables

FORCE (https://force.nutrition.tufts.edu/) was formed within the framework of the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium fatty acid working group (26,27) to assess the relationships of fatty acid biomarkers with health outcomes. For this analysis, cohorts who were members of FORCE as of May 2018 were invited to participate. CASS was invited due to an existing collaboration with an expert within FORCE. Included cohorts had data from participants aged 18 years or older on levels of blood or adipose tissue 1) long-chain n-3 PUFAs (ALA, EPA, and DHA) and 2) n-6 PUFAs (AA and LA) and measures of either sleep duration and/or difficulty falling asleep. Levels of DPA were also evaluated if available but not required. In total, 12 studies had available data and agreed to participate (Table 1). All studies obtained institutional review board approval and informed consent from participants. The pooled analysis was approved by the Clinical Research Ethics Board at the University of British Columbia (H18-01641).

Details of participating cohorts, study participants, fatty acid assessment, and methods for ascertainment of sleep duration and difficulty falling sleeping are presented in the Supplementary Text, Supplementary Table 1 and Supplementary Figure 1. Briefly, fatty acid concentrations were assessed with gas chromatography in each cohort in one or more lipid compartments including red blood cells (RBC), plasma phospholipids (PL), cholesterol esters (CE), total plasma/serum, or adipose tissue (AT). PUFA levels in each cohort were expressed as a percent of total fatty acids in the lipid pool analyzed.

Sleep duration was determined via standardized questionnaires in each cohort (Supplementary Text) using similar methods (e.g., ‘How many hours do you usually sleep per
night?’), with the exception of the CASS that used a self-reported sleep diary. Sleep duration was categorized as short (≤6 hours), normal 7-8 hours (reference) or long (9+ hours), based on existing evidence on sleep duration and health (28,29) and the corresponding recommendations from the American Academy of Sleep Medicine (30) and National Sleep Foundation (15). The upper limit of 8 hours for normal sleep was utilized based on the National Sleep Foundation recommendation for ages 65 and older, as 6 of 10 cohorts assessing sleep duration had a mean age greater than 65. That said, the mean sleep duration within the long sleep category approached or exceeded 9 hours in most cohorts, for example a mean (SD) of 9.79 (1.37) hours in the Finnish Diabetes Prevention Study (Table 1). Difficulty falling sleeping was self-reported by questionnaire with similar methods (e.g., ‘Do you have difficulties falling asleep in the evening?’ and categorized as yes or no (Supplementary methods). The mean sleep duration and prevalence of difficulty falling sleeping is provided in Table 1 along with other cohort descriptors.

Statistical analysis in individual studies

Prior to invitations of cohorts, a standardized analysis protocol was developed, approved by the FORCE central committee, and provided to each participating cohort. The protocol pre-specified the exposures, outcomes, relevant covariates, effect modifiers, and statistical methods. Each cohort subsequently performed de novo, individual-level statistical analysis according to this protocol. Study-specific approaches were permitted for modelling covariates (e.g., number of education categories, case deletion for missing covariates), depending on availability and prior established cohort-specific approaches. Cohort-specific results were entered into a standardized form and compiled centrally; the results were then pooled using meta-analysis.
The primary exposure variables were the n-3 PUFAs (ALA, EPA, DPA, DHA and the sum of EPA+DPA+DHA which was considered a biomarker of fatty fish intake), whereas the n-6 PUFAs (LA and AA) were secondary. Although n-3 and n-6 PUFAs represent distinct fatty acid classes, they share desaturases and elongases in their biosynthesis pathways. Pearson correlation coefficients were calculated between individual PUFAs in each cohort. Multinomial logistic regression models were fitted to data for 1) sleep duration, comparing short and long sleep to normal and 2) difficulty falling sleeping, comparing those who reported difficulty to those who did not. PUFAs were evaluated as a continuous linear variable in a unit of the study-specific interquintile range (IQR, i.e., the difference between the midpoint of the top and bottom quintiles, the 90th and 10th percentiles) and, in separate models, as study-specific quintiles as indicator variables (quintile 1 as referent) to assess potential nonlinear associations.

Model 1 included age, sex, field site if applicable, race (White or non-White), education (<high school, high school graduate, college or higher), occupation (clerical or other), physical activity (kcal/week), smoking (never, former or current), alcohol consumption (servings/week), prevalent hypertension (treated or self-reported), prevalent dyslipidaemia (treated or self-reported), prevalent coronary heart disease, body mass index (BMI) and waist circumference. As dietary behaviors may confound associations between PUFAs and sleep, model 2 further adjusted for melatonin use (yes/no) and diet related variables: fish oil use (yes/no), fish/seafood consumption (servings/week), and the healthy eating index (HEI) or fruit and vegetable consumption (servings/week) if HEI was unavailable. The HEI is an indication of overall diet quality of which ‘seafood and plant proteins’ and fatty acids ‘(polyunsaturated fatty acids (PUFAs) + monounsaturated fatty acids)/saturated fatty acids’ are components comprising a maximum of 15 out of 100 points (31). Study-specific measures of interaction by age (<60 years
or ≥ 60 years), sex (male or female), and BMI (<30kg/m² or ≥30kg/m²) using model 1 were obtained, where possible, depending on cohort demographics.

**Meta-analysis**

Study-specific regression coefficients and standard errors were pooled with an inverse-variance weighted meta-analysis to estimate summary odds ratios (ORs) and corresponding confidence intervals (CIs) per IQR or from quintile comparisons. Linear trends across quintiles were determined by inverse-variance weighted meta-regression. Overall heterogeneity was assessed using the I² (32) and considered low if <35% and moderate if 36-69% (27). Interactions were tested by pooling cohort-specific coefficients of crossproduct terms from model 1 in meta-analysis. As interaction analyses were exploratory, we corrected for multiple testing for these with alpha<0.002 (0.05; 7 PUFA variables; 3 potential effect modifiers). Meta-analyses were performed using R version 3.4.3 (R Foundation for Statistical Computing, Vienna, Austria) and Stata 14.2 (StataCorp LLC, College Station, Texas).

**RESULTS**

Across the 12 participating cohorts, the mean age ranged from 48 to 80 years, and overall age from 35 to 96 years (Table 1). Two cohorts recruited only males, one only females, and nine, both sexes. Average BMI ranged from 23 to 31 kg/m². Most studies recruited predominantly white participants, although meaningful numbers of non-white participants were included in the Women’s Health Initiative-Memory Study (12.4% non-white), Singapore Chinese Health Study (100% Chinese), CASS (30.4% African American, 26.6% Hispanic, 21.9% Asian) and the
Cardiovascular Health Study (15.9% non-white). Ten studies had information on sleep duration (total N=18,791), and eight on difficulty falling sleeping (N=12,500).

Mean (SD) levels and 10th and 90th percentiles for LA, AA, ALA, EPA, DPA, DHA and EPA+DPA+DHA are presented in Supplementary Table 2. PUFA levels within a given compartment (e.g. plasma phospholipids) were largely similar across cohorts with the exception of AGES-R in Iceland, in which cod liver oil use was prevalent, where long-chain n-3 PUFA levels were higher. PUFAs levels varied by lipid compartments and were typical of levels reported in previous studies (27,33,34). Correlations between PUFAs in each cohort are provided in Supplementary Tables 3a and b. In general, LA was inversely associated with n-3 PUFAs while EPA, DPA, DHA and EPA+DPA+DHA were moderately correlated.

**PUFA levels and short sleep duration**

In pooled analyses per IQR, no significant associations between short sleep and any PUFAs were observed in model 1 (Figure 1) or model 2 (Supplementary Figure 2). Heterogeneity was minimal, except moderate for AA (I²=49%). Similarly, pooled analyses of quintiles did not reveal any statistically significant associations (Figure 2), although ORs between very long-chain n-3 PUFA and short sleep tended to be <1.0. No linear trends across quintiles were observed in model 1 or 2 (p>0.05 for all).

**PUFA levels and long sleep duration**

When evaluating long sleep duration (9+ hours), summed levels of very long-chain n-3 PUFA (EPA+DPA+DHA) were associated with lower risk, with an OR per IQR of 0.86 (0.75, 0.99) (Figure 3). Associations of individual very-long chain n-3 PUFA were similar, for
example, the OR per IQR of DHA was 0.86 (0.74, 1.00). Heterogeneity was moderate, with $I^2$ 43.5% for EPA, 58.7% for DHA and 52.7% for EPA+DPA+DHA. Pooled analyses comparing quintiles as indicator categories were consistent with these results, with statistically significant inverse associations across quintiles of EPA, DHA and EPA+DPA+DHA with long sleep duration (Figure 4). For example, the OR (95% CI) for DHA was 0.78, 95% CI 0.65, 0.95 and for EPA+DPA+DHA, 0.76 (0.63, 0.93). Associations were modestly attenuated with additional adjustment for dietary intake and sleep measures in model 2. Linear trends across quintiles for EPA and DHA did not meet statistical significance in model 2 (p=0.09 and p=0.08). No significant associations were seen between n-6 PUFAs and long sleep in model 1 (Figure 3) or model 2 (Supplementary Figure 3).

Difficulty falling sleeping

Higher levels of ALA were associated with a borderline lower risk of difficulty falling sleeping in model 1 (OR per IQR 0.91, 95% CI 0.84, 1.00), but this was attenuated in model 2 (Supplementary Figure 4). No other significant associations between PUFAs and difficulty falling sleeping were observed (Figure 5 and Supplementary Figure 3). Heterogeneity between studies was minimal, highest for ALA ($I^2=33.9$%). No linear trends across quintiles were observed (p>0.05 for all). In quintile analyses, significant associations were generally not identified, although ORs for higher levels of DHA were <1.0 (Figure 6).

Exploratory analyses of effect modification

There was little evidence that the relationship between PUFA levels and sleep varied according to difference in age, sex or BMI (P-interaction=NS for each).
DISCUSSION

Based on harmonized, de novo, individual-level analyses and pooling across 12 studies from 5 countries, higher blood/tissue levels of EPA+DPA+DHA and DHA alone were associated with lower odds of long sleep duration. This was particularly evident when comparing the highest versus lowest quintiles of these n-3 PUFAs. In contrast, no significant associations were identified with short sleep, difficulty falling asleep, or for the plant-derived n-3 ALA or the n-6 LA or AA. These findings, in a well powered, biomarker assessed, diverse study suggest a specificity of association for very long-chain n-3 PUFA biomarkers and long sleep duration; and to our knowledge, represent the most comprehensive examination to-date of the associations between circulating PUFA and measures of sleep.

The lower likelihood of long sleep duration among individuals with higher very long chain n-3 PUFAs may suggest potential positive effects on sleep consolidation/quality. Although sleep duration is one of the most widely used parameter of sleep quality, it is just one dimension of sleep which has other dimensions such as timing and regularity (15). It is unclear why very long-chain n-3 PUFAs were not associated with short sleep duration. It is possible our definition of short sleep duration (<7 hours) versus an alternative definition (e.g. <6 hours) may have altered risk associations. Our findings are, however, supported by a recent randomized trial of either DHA, EPA or placebo on sleep in 84 healthy young adults that found DHA supplementation produced shorter sleep latency (time to sleep onset) and greater sleep efficiency (time asleep/time in bed) versus placebo, while a trend towards greater sleep efficiency with EPA supplementation was also observed (35).
Preclinical evidence provides biologic plausibility supporting these findings through direct or downstream effects of PUFA. DHA has an important role in the pineal gland, which produces melatonin and modulates sleep/wake cycles (36). Fatty acid metabolites (prostaglandin D2, anandamide and 2-arachidonyl glycerol) are also involved in sleep/wake regulation (36). Melatonin production in mice deficient in DHA or with a low DHA:AA ratio in the brain is dysregulated and sleep/wake cycles are disturbed (19,37,38). EPA and DHA may also impact sleep via serotonin. Both fatty acids play a role in serotonin regulation (39), and the serotonergic system has been shown to play a critical role in sleep initiation and sleep maintenance (40). While AA has also been hypothesized to be involved in sleep-wake modulation (41,42), our findings do not suggest a prominent role of AA (or LA) with sleep duration or difficulty falling sleeping.

We found little evidence for associations between ALA, LA, or AA and sleep duration or difficulty falling sleeping. ALA conversion to EPA and DHA is limited (43). Correlations between ALA, EPA, DPA and DHA within individual cohorts in this pooling project were generally modest and inconsistent. Findings therefore suggest a specificity of association for very long-chain n-3 PUFA, that may be related to sleep through biological mechanisms unrelated to ALA. n-6 PUFA were also not associated with sleep measures, in contrast to inverse associations between LA levels with other health outcomes such as cardiovascular disease, cardiovascular mortality and ischemic stroke (44). The scarcity of interventional and observational studies makes it difficult to draw firm conclusions in this area. Our results highlight the need for a greater understanding of biological mechanisms in pre-clinical and clinical models which consider impacts of PUFA and PUFA metabolites to provide context to our findings.
The interaction analyses showed lack of statistically significant effects of sex, age or BMI on associations of PUFAs with sleep duration, and difficulty falling sleeping. This suggests results are not meaningfully different between men and women or across age groups and body weight categories.

Our study has several strengths. Our collaborative pooling of de novo analyses across multiple international cohorts of sleep duration (including a total of 18,791 participants) and difficulty falling sleeping (including a total of 12,500 participants) provides by far the largest assessment to-date of PUFA biomarkers and sleep, increasing both generalisability and statistical power. Cohorts spanned five countries and included populations with diverse background diets, environmental settings and lifestyle practices, making it less likely that any single confounder would explain our results, and increasing generalizability of our findings. Pooling of de novo, individual-level analyses offers many benefits over meta-analyses of published studies, including direct standardization of exposures, outcomes, covariables, and statistical methods, which reduces bias and heterogeneity arising from to methodological variations. An additional strength of our approach is the reduced risk for publication bias. Indeed, none of the studies included here have previously published on PUFAs and sleep and would thus not be included in publication-based meta-analyses. Biomarker assessment of PUFAs reflect both diet and metabolism and are not influenced by misreporting of dietary intake.

There are also potential limitations to our study. Although models adjusted for major potential confounders that influence sleep including age, chronic disease, and BMI, residual confounding may still exist. For example, individuals who have long sleep may have differing medical, occupational, or familial characteristics. However, the findings were generally consistent across populations with diverse demographics and health characteristics and were
present despite adjustment for a range of demographic, socioeconomic and health variables in models. Power to detect potential sources of heterogeneity such as lipid fraction and race/ethnicity was limited, requiring further research. While blood and solid tissue may have differing PUFA pharmacokinetics, all studies on sleep duration used blood levels, and for difficulty falling asleep, only one study (~6% weight) used tissue levels. Findings excluding that study (ULSAM-70) did not appreciably change findings. Further, heterogeneity was generally low to moderate. Power of interaction analyses were subject to demographics and sampling of individual cohorts; for example, distributions of age in different cohorts limited assessment of interaction by the same age threshold across all cohorts. The cross-sectional nature of the analyses precludes determination of the temporal direction of the associations: that is, very long chain n-3 PUFA levels could physiologically contribute to disordered sleep patterns, or individuals with long sleep could consume less very long chain n-3 PUFAs. Sleep duration was self-reported in most cohorts, which may cause misclassification of sleep duration (45) and attenuate findings toward the null. As well, difficulty falling asleep was assessed using a single question which undoubtedly does not adequately capture any nuances in this dimension of sleep. It is thus likely that our results are conservative and may be biased towards the null.

CONCLUSIONS

In this large, biomarker-based, pooling project including 12 large studies from five nations, individuals with lower levels of very long-chain n-3 PUFAs were more likely to have sleep that exceeds current recommended levels. These findings highlight the importance of continued study of very long-chain n-3 PUFAs and sleep given the health implications of poor sleep. There is
also a need to determine the temporality of associations and to further understand the potential underlying biological mechanisms.

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Authors’ contributions to the manuscript: MD, WSH, MAB, MS, NT, VDdM, EL, JKV and RAM analyzed data, RAM designed the research question and approach, RAM wrote the paper, RAM had primary responsibility for final content. WSH, NT, DS, RNL, and DM made substantial contributions to the paper. NT, LD, WSH, DM, gave substantial input on the analytical protocol. All authors read and approved the final manuscript.

Data Sharing: Data described in the manuscript, code book, and analytic code will be made available upon request pending approval from FORCE.
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Figure 1. Forest plot of associations between n-3 and n-6 polyunsaturated fatty acids per
interquintile range and short sleep duration

**Legend.** Odds ratios (ORs) and 95% confidence intervals (95% CI) per interquintile range defined as 90th minus 10th percentiles of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models for Model 1, N=18,791. Each cohort-specific association was assessed with multivariable regression models adjusted for sex, age, field site (if applicable), race, income, education, occupation, smoking status, physical activity, alcohol consumption, prevalent dyslipidemia, prevalent hypertension, prevalent coronary heart disease, triglycerides, body-mass index, and waist circumference. POEM, and SCHS only have data for combined EPA+DHA, as DPA data were not measured. Abbreviations: AA; arachidonic acid, AGES-R; Age, Gene/Environment Susceptibility-Reykjavik, ALA; alpha-linolenic acid, CASS; Chicago Area Sleep Study, CE; cholesterol ester, CHS; Cardiovascular Health Study, DHA; docosahexaenoic acid, DPS; Finnish Diabetes Prevention Study, DPA; docosapentaenoic acid, EPA; eicosapentaenoic acid, FHS; Framingham Heart Study, KIHD; Kuopio Ischaemic Heart Disease Risk Factor Study, LA; linoleic acid, PIVUS; Prospective Investigation of the Vasculature in Uppsala Seniors, PL; phospholipid, POEM; Prospective investigation of Obesity, Energy and Metabolism, RBC; red blood cell, SCHS; Singapore Chinese Health Study, WHIMS; Women’s Health Initiative-Memory Study.
Figure 2. Forest plot of associations between quintiles of n-3 and n-6 polyunsaturated fatty acids and short sleep

Legend. Odds ratios (ORs) and 95% confidence intervals (95% CI) per quintile of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models, N=18,791. Model 1 included age, sex, field site, race, education, occupation, physical activity, smoking, alcohol use, prevalent hypertension (treated or self-reported), prevalent dyslipidemia (treated or self-reported), prevalent coronary heart disease, BMI and waist circumference. Model 2 further adjusted for dietary and sleep-related variables: melatonin use, fish/seafood consumption, and HEI or fruit and vegetable consumption if HEI was unavailable.

Abbreviations: AA; arachidonic acid, AGES-R; Age, Gene/Environment Susceptibility-Reykjavik, ALA; alpha-linolenic acid, CASS; Chicago Area Sleep Study, CE; cholesterol ester, CHS; Cardiovascular Health Study, DHA; docosahexaenoic acid, DPS; Finnish Diabetes Prevention Study, DPA; docosapentaenoic acid, EPA; eicosapentaenoic acid, FHS; Framingham Heart Study, KIHD; Kuopio Ischaemic Heart Disease Risk Factor Study, LA; linoleic acid, PIVUS; Prospective Investigation of the Vasculature in Uppsala Seniors, PL; phospholipid,
POEM; Prospective investigation of Obesity, Energy and Metabolism, RBC; red blood cell, SCHS; Singapore Chinese Health Study, WHIMS; Women’s Health Initiative-Memory Study
Figure 3. Forest plot of associations between n-3 and n-6 polyunsaturated fatty acids per interquintile range and long sleep
Legend. Odds ratios (ORs) and 95% confidence intervals (95% CI) per interquintile range defined as 90th minus 10th percentiles of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models for Model 1, N=18,791. Each cohort-specific association was assessed with multivariable regression models adjusted for sex, age, field site (if applicable), race, income, education, occupation, smoking status, physical activity, alcohol consumption, prevalent dyslipidemia, prevalent hypertension, prevalent coronary heart disease, triglycerides, body-mass index, and waist circumference. POEM, and SCHS only have data for combined EPA+DHA, as DPA data are not available.

Abbreviations: AA; arachidonic acid, AGES-R; Age, Gene/Environment Susceptibility-Reykjavik, ALA; alpha-linolenic acid, CASS; Chicago Area Sleep Study, CE; cholesterol ester, CHS; Cardiovascular Health Study, DHA; docosahexaenoic acid, DPS; Finnish Diabetes Prevention Study, DPA; docosapentaenoic acid, EPA; eicosapentaenoic acid, FHS; Framingham Heart Study, KIHD; Kuopio Ischaemic Heart Disease Risk Factor Study, LA; linoleic acid, PIVUS; Prospective Investigation of the Vasculature in Uppsala Seniors, PL; phospholipid, POEM; Prospective investigation of Obesity, Energy and Metabolism, RBC; red blood cell, SCHS; Singapore Chinese Health Study, WHIMS; Women’s Health Initiative-Memory Study
Figure 4. Forest plot of associations between quintiles of n-3 and n-6 polyunsaturated fatty acids and long sleep duration

Legend. Odds ratios (ORs) and 95% confidence intervals (95% CI) per quintile of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models, N=18,791.

Model 1 included age, sex, field site, race, education, occupation, physical activity, smoking, alcohol use, prevalent hypertension (treated or self-reported), prevalent dyslipidemia (treated or self-reported), prevalent coronary heart disease, BMI and waist circumference. Model 2 further adjusted for dietary and sleep-related variables: melatonin use, fish/seafood consumption, and HEI or fruit and vegetable consumption if HEI was unavailable.

Abbreviations: AA; arachidonic acid, AGES-R; Age, Gene/Environment Susceptibility-Reykjavik, ALA; alpha-linolenic acid, CASS; Chicago Area Sleep Study, CE; cholesterol ester, CHS; Cardiovascular Health Study, DHA; docosahexaenoic acid, DPS; Finnish Diabetes Prevention Study, DPA; docosapentaenoic acid, EPA; eicosapentaenoic acid, FHS; Framingham Heart Study, KIHD; Kuopio Ischaemic Heart Disease Risk Factor Study, LA; linoleic acid, PIVUS; Prospective Investigation of the Vasculature in Uppsala Seniors, PL; phospholipid,
POEM; Prospective investigation of Obesity, Energy and Metabolism, RBC; red blood cell,
SCHS; Singapore Chinese Health Study, WHIMS; Women’s Health Initiative-Memory Study
Figure 5. Forest plot of associations between n-3 and n-6 polyunsaturated fatty acids per interquintile range and difficulty sleeping
Legend. Odds ratios (ORs) and 95% confidence intervals (95% CI) per interquintile range defined as 90th minus 10th percentiles of circulating or tissue PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models for Model 1, N=12,500. Each cohort-specific association was assessed with multivariable regression models adjusted for sex, age, field site (if applicable), race, income, education, occupation, smoking status, physical activity, alcohol consumption, prevalent dyslipidemia, prevalent hypertension, prevalent coronary heart disease, triglycerides, body-mass index, and waist circumference. POEM, and ULSAM-50 only have data for combined EPA+DHA, as DPA was not measured.

Abbreviations: AA; arachidonic acid, AGES-R; Age, Gene/Environment Susceptibility-Reykjavik, ALA; alpha-linolenic acid, CASS; Chicago Area Sleep Study, CE; cholesterol ester, CHS; Cardiovascular Health Study, DHA; docosahexaenoic acid, DPS; Finnish Diabetes Prevention Study, DPA; docosapentaenoic acid, EPA; eicosapentaenoic acid, FHS; Framingham Heart Study, KIHD; Kuopio Ischaemic Heart Disease Risk Factor Study, LA; linoleic acid, PIVUS; Prospective Investigation of the Vasculature in Uppsala Seniors, PL; phospholipid, POEM; Prospective investigation of Obesity, Energy and Metabolism, RBC; red blood cell, SCHS; Singapore Chinese Health Study, WHIMS; Women’s Health Initiative-Memory Study.
Figure 6. Forest plot of associations between quintiles of n-3 and n-6 polyunsaturated fatty acids and difficulty sleeping

Legend. Odds ratios (ORs) and 95% confidence intervals (95% CI) per quintile of circulating or tissue PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models, N=12,500. Model 1 included age, sex, field site, race, education, occupation, physical activity, smoking, alcohol use, prevalent hypertension (treated or self-reported), prevalent dyslipidemia (treated or self-reported), prevalent coronary heart disease, BMI and waist circumference. Model 2 further adjusted for dietary and sleep-related variables: melatonin use, fish/seafood consumption, and healthy eating index (HEI) or fruit and vegetable consumption if HEI was unavailable. Abbreviations: AA; arachidonic acid, AGES-R; Age, Gene/Environment Susceptibility-Reykjavik, ALA; alpha-linolenic acid, CASS; Chicago Area Sleep Study, CE; cholesterol ester, CHS; Cardiovascular Health Study, DHA; docosahexaenoic acid, DPS; Finnish Diabetes Prevention Study, DPA; docosapentaenoic acid, EPA; eicosapentaenoic acid, FHS; Framingham Heart Study, KIHD; Kuopio Ischaemic Heart Disease Risk Factor Study, LA; linoleic acid, PIVUS; Prospective Investigation of the Vasculature in Uppsala Seniors, PL;
phospholipid, POEM; Prospective investigation of Obesity, Energy and Metabolism, RBC; red blood cell, SCHS; Singapore Chinese Health Study, WHIMS; Women’s Health Initiative-Memory Study
Table 1. Description of the 12 cohorts that participated in the pooled analysis of n-3 and n-6 PUFA levels and sleep

| Study     | Country          | Year(s) of blood sample | N    | Age (years), mean (SD) | Sex, % women | BMI (kg/m²), mean (SD) | Triglycerides (mg/dL), mean (SD) | Lipid fraction | Sleep duration (hours), mean (SD) or % | Difficulty falling asleep, % yes |
|-----------|------------------|-------------------------|------|------------------------|--------------|------------------------|----------------------------------|----------------|----------------------------------------|---------------------------------|
| AGES-R    | Iceland          | 2002-2006               | 1697 | 76.7 (5.50)            | 44.8         | 27.2 (4.31)            | 109 (58.4)                        | PL             | 62.3                                   | 20.1                            |
| CASS      | US               | 2009-2011               | 618  | 48.1 (8.30)            | 56.7         | 26.6 (4.60)            | 116 (72.4)                        | plasma         | 6.8 (1.2)                              | 30.4                            |
| CHS       | US               | 1998-1999               | 2566 | 79.7 (4.50)            | 60.9         | 26.6 (4.50)            | 138 (71.4)                        | PL             | 7.3 (1.5)                              | 29.0                            |
| FHS       | US               | 2005-2008               | 2562 | 66.2 (8.83)            | 44.8         | 28.2 (5.33)            | 118 (69.5)                        | RBC            | 7.1 (1.2)                              | 39.0                            |
| WHI-MS    | US               | 1995                    | 6330 | 70.1 (3.84)            | 100          | 28.4 (5.62)            | NA                               | RBC            | 35.9                                   | NA                              |
| DPS       | Finland          | 1993-1996               | 393  | 55.4 (7.14)            | 67.9         | 31.1 (4.69)            | NA                               | serum          | 8.8 (1.8)                              | NA                              |
| KIHD      | Finland          | 1998-2001               | 1694 | 62.8 (6.50)            | 52.3         | 27.9 (4.50)            | 113 (62.8)                        | serum          | 7.4 (0.8)                              | 25.8                            |
| PIVUS     | Sweden           | 2001-2004               | 942  | 70                      | 49.4         | 27.0 (4.23)            | 113 (53.3)                        | CE, PL         | 7.1 (1.1)                              | NA                              |
| POEM      | Sweden           | 2010-2016               | 501  | 50                      | 50.5         | 26.4 (4.26)            | 105 (78.4)                        | CE             | 7.1 (0.9)                              | 10.6                            |
| ULSAM-50  | Sweden           | 1970-1973               | 2009 | 49.7 (0.59)            | 0            | 25.1 (3.20)            | 175 (103)                         | CE             | NA                                     | 15.2                            |
| ULSAM-70  | Sweden           | 1991-1995               | 853  | 70.9 (0.62)            | 0            | 26.4 (3.40)            | 128 (67.1)                        | AT             | NA                                     | 10                              |
| SCHS      | Singapore        | 1994-2005               | 1488 | 66 (7.77)              | 35.3         | 23 (3.02)              | 145 (60)                          | plasma         | 40.5                                   | NA                              |

NA reflects unavailable data. Sleep duration was measured in categories in WHIMS, AGES-R, and SCHS where % shown represents sleep duration in referent category of 7-8 hours. Abbreviations: AGES-R: Age, Gene/Environment Susceptibility Study-Reykjavik; AT, adipose tissue; CASS, Chicago Area Sleep Study; CE, cholesterol esters; CHS, Cardiovascular Health Study, DPS, Finnish Diabetes Prevention Study, FHS, Framingham Heart Study; KIHD, Kuopio Ischaemic Heart Disease; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; PL, phospholipids; POEM, Prospective Investigation of Obesity, Energy and Metabolism; RBC, red blood cells; SCHS, Singapore Chinese Health Study; ULSAM-50, Uppsala Longitudinal Study of Adult Men-50; ULSAM-70, Uppsala Longitudinal Study of Adult Men-70; US, United States, WHI-MS, Women’s Health Initiative-Memory Study. No SDs are shown for age in PIVUS or POEM as participants were purposefully recruited to be 70 and 50 years of age.