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PUFA ω-3 and ω-6 biomarkers and sleep: a pooled analysis of cohort studies on behalf of the Fatty Acids and Outcomes Research Consortium (FORCE)

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ABSTRACT

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

Objectives: We sought to assess associations between biomarkers of n-3 and n-6 PUFA intake with self-reported sleep duration and difficulty falling asleep in the Fatty Acids and Outcome Research Consortium.

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

Results: In pooled analysis adjusted for sociodemographic characteristics and health status, participants with higher very long-chain n-3 PUFAs were less likely to have long sleep duration. In the top compared with the bottom quintiles, the multivariable-adjusted ORs (95% CIs) for long sleep were 0.78 (95% CI: 0.65, 0.95) for DHA and 0.76 (95% CI: 0.63, 0.93) for EPA + DPA + DHA. Significant associations for ALA and n-6 PUFA with short sleep duration or difficulty falling asleep were not identified.

Conclusions: Participants with higher concentrations 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 relation. These novel findings across 12 cohorts highlight the need for experimental and biological assessments of very long-chain n-3 PUFAs and sleep duration. *Am J Clin Nutr* 2022;115:864–876.

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

Introduction

A number of epidemiologic studies show that short sleep (≤6 h/d) is associated with a variety of physical impairments (1) and 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 relations have been observed among people who report long sleep duration (≥9 h/d) (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 y sleep ≥7 h per night (14). The National Sleep Foundation has a similar lower-limit recommendation but places an upper limit of 8 h for people aged ≥65 and 9 h for people aged 18–64 y (15). According to national data from the United States, 35% of adults report insufficient sleep (≤6 h) (16), and sleep deprivation has been described as a major public health problem by the CDC (17).

Certain nutrients may have physiologic effects on sleep regulation, particularly n-3 and n-6 PUFAs. DHA is important for sleep regulation (18) through its a role in regulating melatonin production (19). A study of 63 obese adults with sleep apnea found that higher tissue concentrations 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 concentrations of DHA and lower DHA:arachidonic acid (AA, 20:4n-6) ratios at baseline had more sleep disturbances. DHA supplementation for 16 wk 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 compared with lower plasma DHA (across quartiles) was associated with 32 min more sleep (23). Oily fish intake was positively associated with sleep quality among 677 adults in Ecuador (24). PUFA (n-6) 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 PUFAs including plant-derived α-linolenic acid (18:3n-3, ALA), seafood-derived, very long-chain 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 relations between n-3 and n-6 PUFA biomarkers and sleep. We hypothesized that lower concentrations of both PUFA families would be associated with suboptimal sleep, including greater risk of short and long sleep and difficulty falling asleep.

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 relations of fatty acid biomarkers with health outcomes. For this analysis, cohorts who were members of FORCE as of May 2018 were invited to participate. The Chicago Area Sleep Study (CASS) cohort was included due to an existing collaboration with an expert within FORCE. Included cohorts had data from participants aged ≥18 y on concentrations in blood or adipose tissue of the following: *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. Concentrations of DPA were also evaluated if available but were 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, and fatty acid assessment and methods for ascertainment of sleep duration and difficulty falling asleep are presented in the **Supplementary Methods**, **Supplementary Table 1**, and **Supplementary Figure 1**. Briefly, fatty acid concentrations were assessed with GC in each cohort in ≥1 lipid compartments, including RBCs, plasma phospholipids, cholesterol esters, total plasma/serum,

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The opinions expressed herein are those of the authors and do not necessarily represent the views of the funding organization. Funding organizations for participating cohorts or investigators had no roles in the collection, analysis, and interpretation of the data; and the decision to submit.

Supplemental Methods, Supplemental Figures 1–4 and Supplemental Tables 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AA, arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility Study-Reykjavik; ALA, α-linolenic acid; CASS, Chicago Area Sleep Study; CHS, Cardiovascular Health Study; DPA, docosapentaenoic acid; DPS, Finnish Diabetes Prevention Study; EPA, eicosapentaenoic acid; FHS, Framingham Heart Study; FORCE, Fatty Acids and Outcomes Research Consortium; HEI, healthy eating index; IQR, interquintile range; 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; 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.

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TABLE 1 Description of the 12 cohorts that participated in the pooled analysis of n-3 and n-6 PUFA concentrations and sleep[1]

Study	Country	Blood sample collection period, y	*n*	Age, y	Sex, female	BMI	Triglycerides, mg/dL	Lipid fraction	Sleep duration, h	Difficulty falling asleep, %
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[2]	Sweden	2001–2004	942	70	49.4	27.0 ± 4.23	113 ± 53.3	CE, PL	7.1 ± 1.1	NA
POEM[2]	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

[1] Values are means ± SDs or percentages unless otherwise indicated. Sleep duration was measured in categories in WHIMS, AGES-R, and SCHS, where percentages shown represent sleep duration in the referent category of 7–8 h. AGES-R, Age, Gene/Environment Susceptibility Study-Reykjavik; AT, adipose tissue; CASS, Chicago Area Sleep Study; CE, cholesterol ester; CHS, Cardiovascular Health Study; DPS, Finnish Diabetes Prevention Study; FHS, Framingham Heart Study; KIHD, Kuopio Ischaemic Heart Disease; NA, not available; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; PL, phospholipid; POEM, Prospective Investigation of Obesity, Energy and Metabolism; SCHS, Singapore Chinese Health Study; ULSAM-50, Uppsala Longitudinal Study of Adult Men-50; ULSAM-70, Uppsala Longitudinal Study of Adult Men-70; WHI-MS, Women's Health Initiative-Memory Study.

[2] No SDs are shown for age in PIVUS or POEM as participants were purposefully recruited to be 70 and 50 y of age.

or adipose tissue. PUFA concentrations in each cohort were expressed as a percentage of total fatty acids in the lipid pool analyzed.

Sleep duration was determined via standardized questionnaires in each cohort (Supplementary Methods) using similar methods (e.g., "How many hours do you usually sleep per night?"), with the exception of the CASS, for which a self-reported sleep diary was used. Sleep duration was categorized as short (≤6 h), normal 7–8 h (reference), or long (9+ h) 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 h for normal sleep was utilized based on the National Sleep Foundation recommendation for individuals aged ≥65 y, as 6 of 10 cohorts assessing sleep duration had a mean age >65 y. That said, the mean sleep duration within the long sleep category was ≥9 h in most cohorts. For example, a mean ± SD of 9.79 ± 1.37 h in the Finnish Diabetes Prevention Study (Table 1). Difficulty falling asleep 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 asleep is provided in Table 1 along with other cohort descriptors.

Statistical analysis in individual studies

Prior to invitation of cohorts, a standardized analysis protocol was developed, approved by the FORCE central committee, and provided to each participating cohort. The protocol prespecified 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 modeling 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 desaturates 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 the following: *1*) sleep duration, comparing short and long to normal sleep, and *2*) difficulty falling asleep 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 nonwhite), 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/wk), prevalent hypertension (treated or self-reported), prevalent dyslipidaemia (treated or self-reported), prevalent coronary artery disease, 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/wk), and the healthy eating index (HEI) or fruit and vegetable consumption (servings/wk) 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 or ≥60 y), sex (male or female), and BMI (in kg/m^2; <30 or ≥30) using model 1 were obtained, where possible, depending on cohort demographics.

Meta-analysis

Study-specific regression coefficients and SEs were pooled with an inverse-variance weighted meta-analysis to estimate summary ORs and corresponding CIs per IQR or from quintile comparisons. Linear trends across quintiles were determined by inverse-variance weighted meta-regression. Overall heterogeneity was assessed using I^2 (32) and considered low if <35% and moderate if 36–69% (27). Interactions were tested by pooling cohort-specific coefficients of cross-product 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) and Stata 14.2 (StataCorp LLC).

Results

Across the 12 participating cohorts, the mean age ranged from 48 to 80 y, and overall age from 35 to 96 y (Table 1). Two cohorts recruited only males, 1 only females, and 9 both sexes. Average BMI ranged from 23 to 31. Most studies recruited predominantly white participants, although meaningful numbers of nonwhite participants were included in the Women's Health Initiative–Memory Study (12.4% nonwhite), Singapore Chinese Health Study (100% Chinese), CASS (30.4% African American, 26.6% Hispanic, 21.9% Asian), and the Cardiovascular Health Study (15.9% nonwhite). Ten studies had information on sleep duration (total $n = 18{,}791$), and 8 on difficulty falling asleep ($n = 12{,}500$).

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

PUFA concentrations 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 ($I^2 = 49\%$). Similarly, pooled analyses of quintiles did not reveal any statistically significant associations (**Figure 2**), although ORs between very long-chain n-3 PUFAs 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 concentrations and long sleep duration

When evaluating long sleep duration (9+ h), summed concentrations of very long-chain n-3 PUFAs (EPA + DPA + DHA) were associated with lower risk, with an OR per IQR of 0.86 (95% CI: 0.75, 0.99) (**Figure 3**). Associations of individual very-long chain n-3 PUFAs were similar; for example, the OR per IQR of DHA was 0.86 (95% CI: 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 (95% CI: 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 asleep

Higher concentrations of ALA were associated with a borderline lower risk of difficulty falling asleep 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 asleep 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 concentrations of DHA were <1.0 (**Figure 6**).

Exploratory analyses of effect modification

There was little evidence that the relation between PUFA concentrations and sleep varied according to differences in age, sex, or BMI (P-interaction for each not significant).

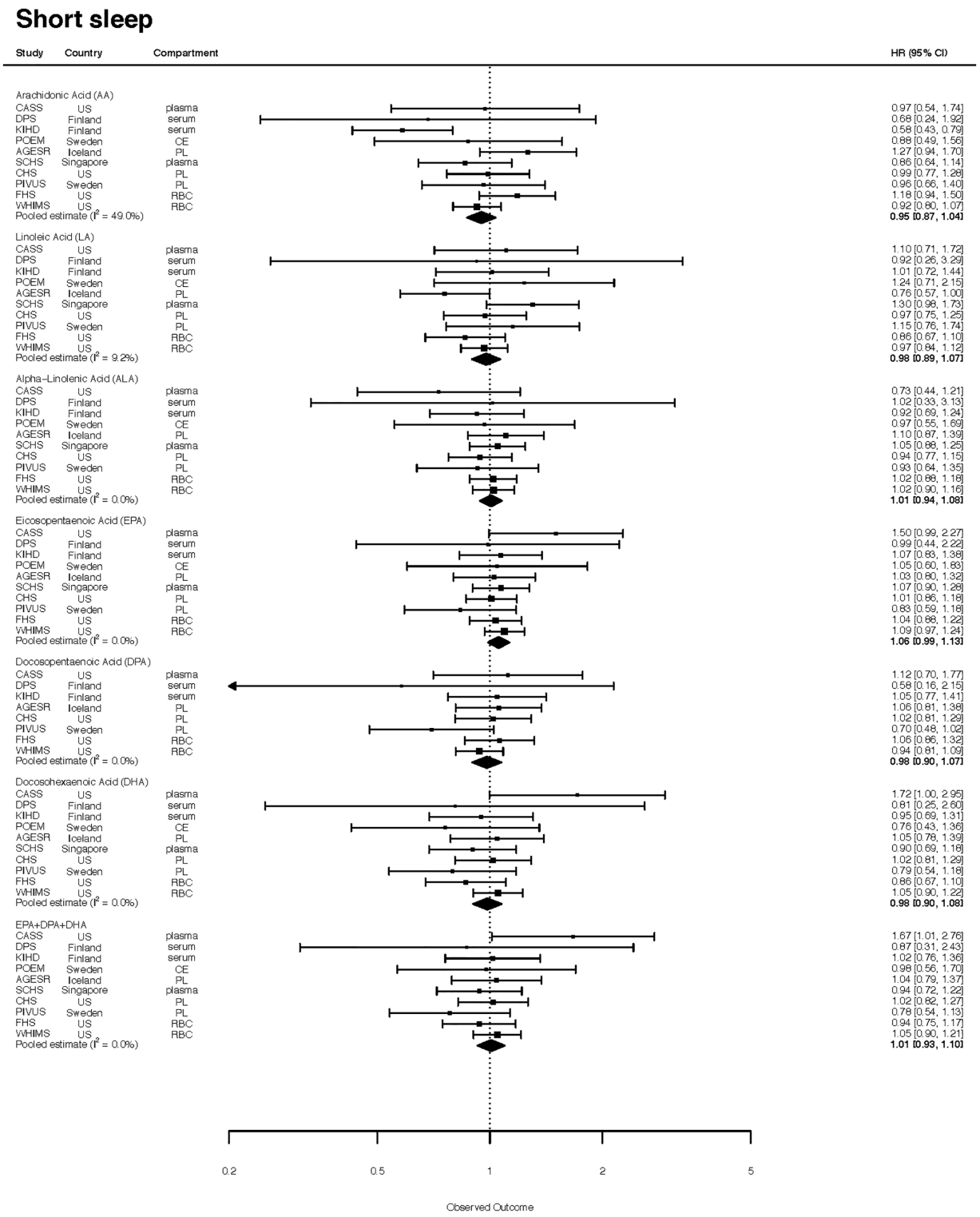

FIGURE 1 Forest plot of associations between n-3 and n-6 PUFAs per IQR and short sleep duration. ORs and 95% CIs per IQR defined as 90th minus 10th percentiles of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models for model 1, $n = 18{,}791$. Each cohort-specific 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 artery disease, triglycerides, BMI, and waist circumference. POEM and SCHS only have data for combined EPA + DHA, as DPA data were not measured. AA; arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility-Reykjavik; ALA, α-linolenic acid; CASS, Chicago Area Sleep Study; CE, cholesterol ester; CHS, Cardiovascular Health Study; DPS, Finnish Diabetes Prevention Study; DPA; docosapentaenoic acid; EPA; eicosapentaenoic acid; FHS, Framingham Heart Study; IQR, interquintile range; 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; SCHS, Singapore Chinese Health Study; WHIMS, Women's Health Initiative-Memory Study.

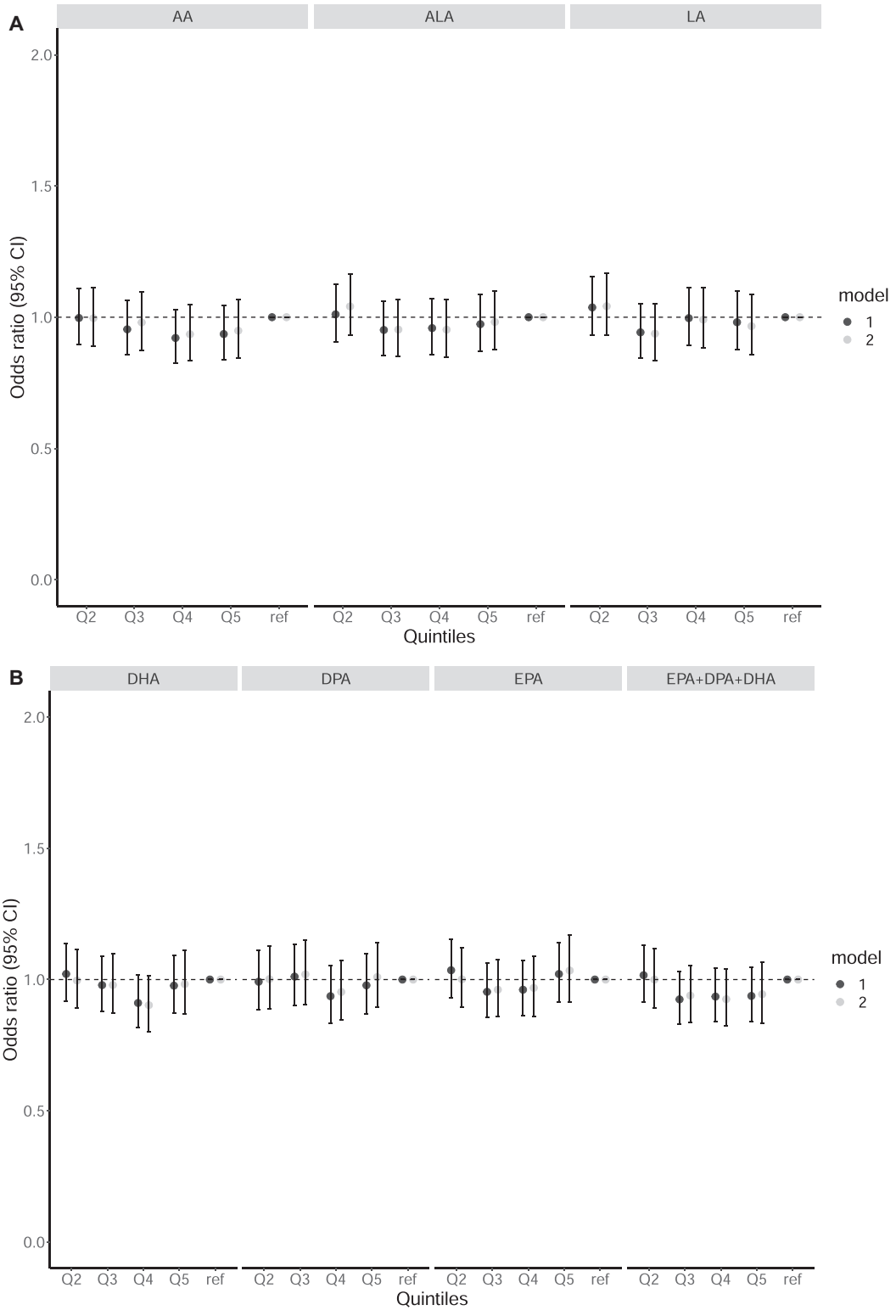

FIGURE 2 Forest plot of associations between quintiles of n-3 and n-6 PUFAs and short sleep. ORs and 95% CIs per quintile of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models, $n = 18{,}791$. Model 1 included age, sex, field site, race, education, occupation, physical activity, smoking, alcohol use, prevalent hypertension (treated or self-reported), prevalent dyslipidemia (treated or self-reported), prevalent coronary artery disease, BMI, and waist circumference. Model 2 was further adjusted for dietary and sleep-related variables: melatonin use, fish/seafood consumption, and HEI or fruit and vegetable consumption if HEI was unavailable. AA, arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility-Reykjavik; ALA, α-linolenic acid; CASS; Chicago Area Sleep Study; CE; cholesterol ester; CHS, Cardiovascular Health Study; DPS, Finnish Diabetes Prevention Study; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FHS, Framingham Heart Study; HEI, healthy eating index; 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; SCHS; Singapore Chinese Health Study; WHIMS, Women's Health Initiative-Memory Study.

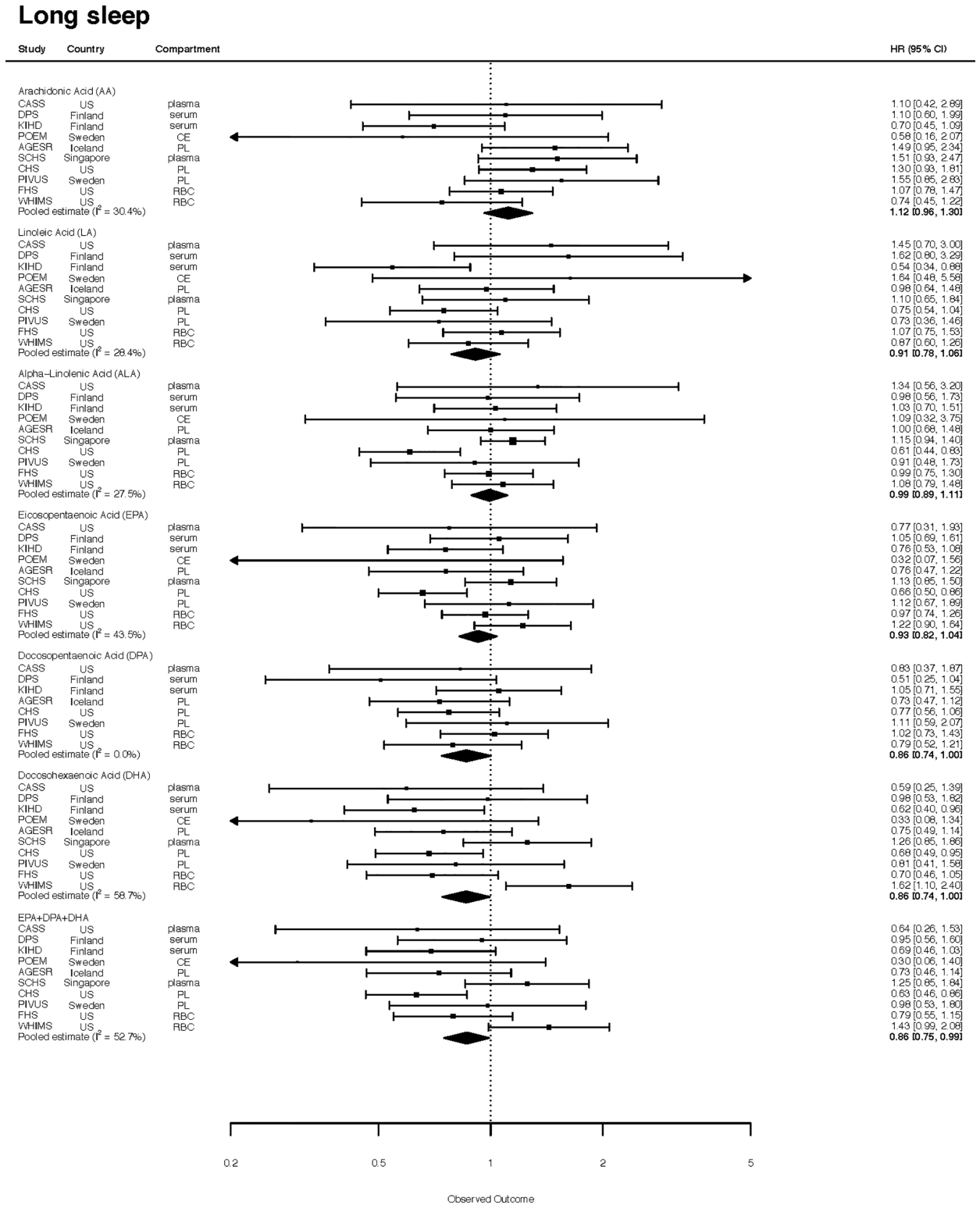

FIGURE 3 Forest plot of associations between n-3 and n-6 PUFAs per IQR and long sleep. ORs and 95% CIs per IQR defined as 90th minus 10th percentiles of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models for model 1, $n = 18{,}791$. Each cohort-specific association 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 artery disease, triglycerides, BMI, and waist circumference. POEM and SCHS only have data for combined EPA + DHA, as DPA data are not available. AA, arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility-Reykjavik; ALA, α-linolenic acid; CASS, Chicago Area Sleep Study; CE; cholesterol ester; CHS, Cardiovascular Health Study; 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; SCHS, Singapore Chinese Health Study; WHIMS, Women's Health Initiative-Memory Study.

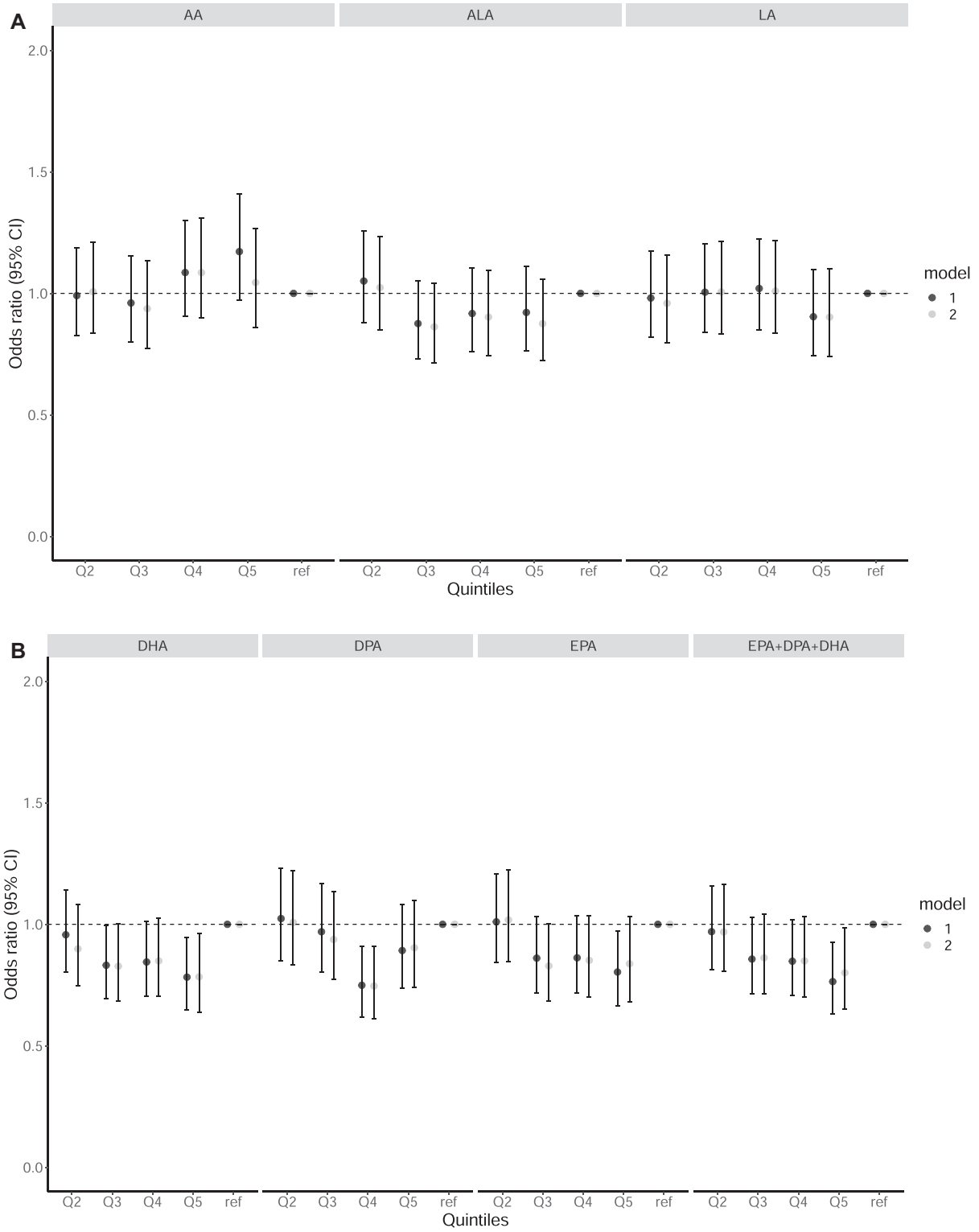

FIGURE 4 Forest plot of associations between quintiles of n-3 and n-6 PUFAs and long sleep duration. ORs and 95% CIs per quintile of circulating PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models, $n = 18{,}791$. Model 1 included age, sex, field site, race, education, occupation, physical activity, smoking, alcohol use, prevalent hypertension (treated or self-reported), prevalent dyslipidemia (treated or self-reported), prevalent coronary artery disease, BMI, and waist circumference. Model 2 was further adjusted for dietary and sleep-related variables: melatonin use, fish/seafood consumption, and HEI or fruit and vegetable consumption if HEI was unavailable. AA, arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility-Reykjavik, ALA, α-linolenic acid; CASS, Chicago Area Sleep Study; CE, cholesterol ester; CHS, Cardiovascular Health Study; DPS, Finnish Diabetes Prevention Study; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FHS, Framingham Heart Study; HEI, healthy eating index; 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; SCHS, Singapore Chinese Health Study; WHIMS, Women's Health Initiative-Memory Study.

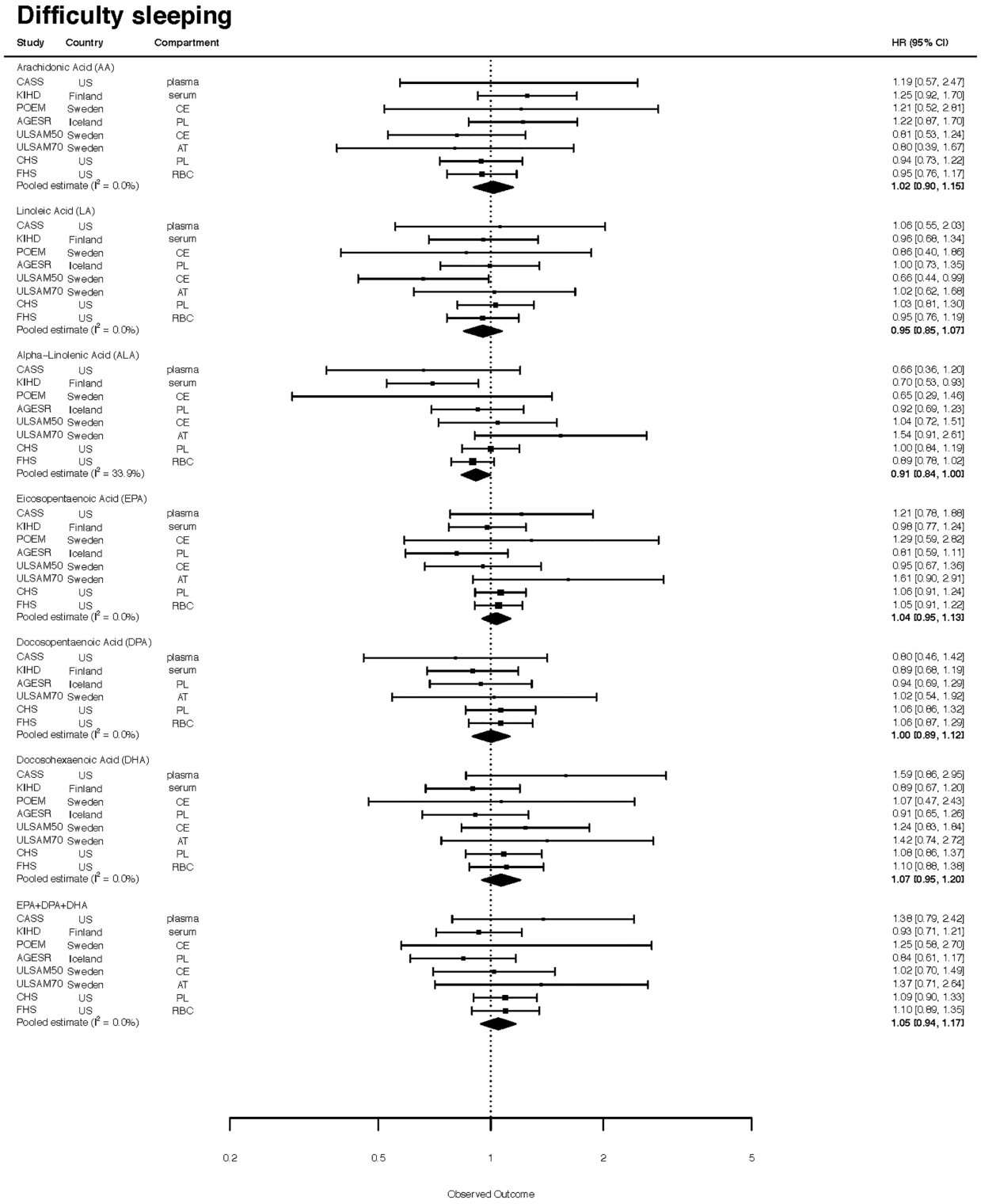

FIGURE 5 Forest plot of associations between n-3 and n-6 PUFAs per IQR and difficulty sleeping. ORs and 95% CIs per IQR defined as 90th minus 10th percentiles of circulating or tissue PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models for model 1, $n = 12{,}500$. Each cohort-specific 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 artery disease, triglycerides, BMI, and waist circumference. POEM and ULSAM-50 only have data for combined EPA + DHA, as DPA was not measured. AA, arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility-Reykjavik; ALA, α-linolenic acid; CASS, Chicago Area Sleep Study; CE, cholesterol ester; CHS, Cardiovascular Health Study; 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; SCHS, Singapore Chinese Health Study; ULSAM-70, Uppsala Longitudinal Study of Adult Men-70; WHIMS, Women's Health Initiative-Memory Study.

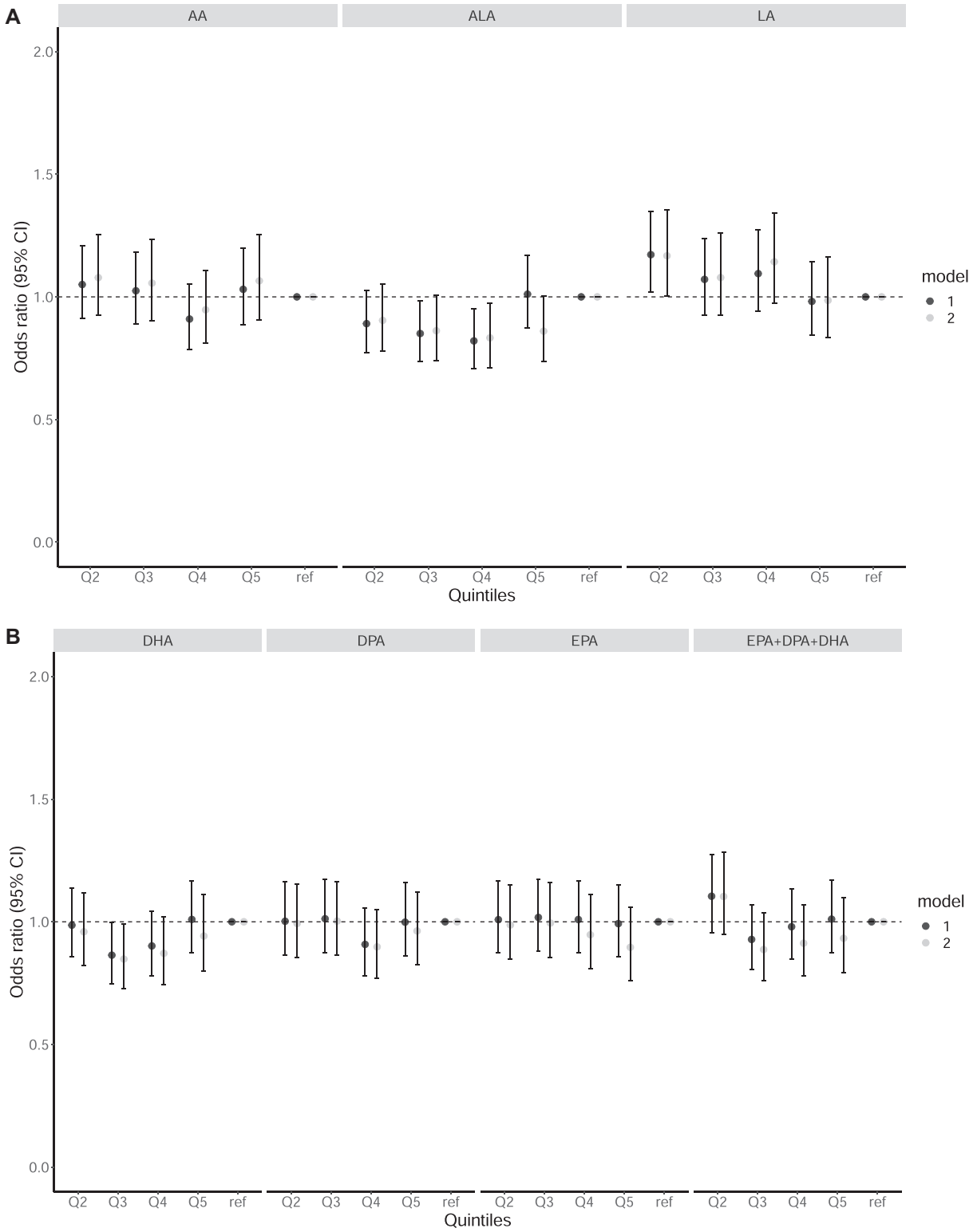

FIGURE 6 Forest plot of associations between quintiles of n-3 and n-6 PUFAs and difficulty sleeping. ORs and 95% CIs per quintile of circulating or tissue PUFA pooled by inverse variance weighted meta-analysis from fixed-effect models, $n = 12{,}500$. Model 1 included age, sex, field site, race, education, occupation, physical activity, smoking, alcohol use, prevalent hypertension (treated or self-reported), prevalent dyslipidemia (treated or self-reported), prevalent coronary artery 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. AA, arachidonic acid; AGES-R, Age, Gene/Environment Susceptibility-Reykjavik; ALA, α-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; HEI, healthy eating index; 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; SCHS, Singapore Chinese Health Study, WHIMS; Women's Health Initiative-Memory Study.

Discussion

Based on harmonized, de novo, individual-level analyses and pooling across 12 studies from 5 countries, higher blood/tissue concentrations of EPA + DPA + DHA and DHA alone were associated with lower odds of long sleep duration. This was particularly evident when comparing the highest with the 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, this study represents the most comprehensive examination to date of the associations between circulating PUFAs and measures of sleep.

The lower likelihood of long sleep duration among individuals with higher concentrations of very long chain n-3 PUFAs may suggest potential positive effects on sleep consolidation/quality. Although sleep duration is 1 of the most widely used parameters of sleep quality, it is just 1 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 h) compared with an alternative definition (e.g., <6 h) may have altered risk associations. Our findings are, however, supported by a recent randomized trial of the effects of either DHA, EPA, or placebo on sleep in 84 healthy young adults that found that DHA supplementation produced shorter sleep latency (time to sleep onset) and greater sleep efficiency (time asleep/time in bed) compared with placebo, while a trend toward greater sleep efficiency with EPA supplementation was also observed (35).

Preclinical evidence provides biologic plausibility supporting these findings through direct or downstream effects of PUFAs. 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 the 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) in sleep duration or difficulty falling asleep.

We found little evidence for associations between ALA, LA, or AA and sleep duration or difficulty falling asleep. 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 PUFAs that may be related to sleep through biological mechanisms unrelated to ALA, and n-6 PUFAs were also not associated with sleep measures, in contrast to inverse associations between LA concentrations 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 preclinical and clinical models which consider impacts of PUFAs and PUFA metabolites to provide context to our findings.

The interaction analyses showed a lack of statistically significant effects of sex, age, or BMI on associations of PUFAs with sleep duration and difficulty falling asleep. This suggests that the 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 asleep (including a total of 12,500 participants) provides by far the largest assessment to date of PUFA biomarkers and sleep, increasing both generalizability and statistical power. Cohorts spanned 5 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 the 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 methodological variations. An additional strength of our approach is the reduced risk for publication bias. Indeed, none of the studies included here have findings previously published on PUFAs and sleep and would thus not be included in publication-based meta-analyses. Biomarker assessment of PUFAs reflects both diet and metabolism and is 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 concentrations, and for difficulty falling asleep, only 1 study (~6% weight) used tissue concentrations. Findings excluding that study (Uppsala Longitudinal Study of Adult Men) 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 concentrations could physiologically contribute to disordered sleep patterns, or individuals with long sleep could consume fewer 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. In addition, difficulty falling asleep was assessed

using a single question, which undoubtedly did not adequately capture any nuances in this dimension of sleep. It is thus likely that our results are conservative and may be biased toward the null.

Conclusions

In this large, biomarker-based pooling project including 12 large studies from 5 nations, individuals with lower concentrations of very long-chain n-3 PUFAs were more likely to have sleep that exceeds the current recommended duration. 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.

The authors' responsibilities were as follows—MD, WSH, MAB, MS, NT, VDdM, EL, JKV, RAM: analyzed data; RAM: designed the research question and approach, wrote the paper, and had primary responsibility for final content; WSH, NT, DS, RNL, DM: made substantial contributions to the manuscript; NT, LD, WSH, DM: gave substantial input on the analytical protocol; and all authors: read and approved the final manuscript.

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Data Availability

Data described in the manuscript, code book, and analytic code will be made available upon request pending approval from the Fatty Acids and Outcomes Research Consortium.

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