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Global variations in omega-3 fatty acid status and omega-6:omega-3 ratios: insights from > 500,000 whole-blood dried blood spot samples

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Abstract

Omega-3 (*n*-3) fatty acids are critical for human health, with the omega-6 (*n*-6) to *n*-3 fatty acid ratio and the omega-3 levels recognized as contributing to the risk of many chronic non-communicable diseases. Direct measurement of fatty acid levels in blood provides the most accurate assessment of fatty acid status and balance, with fingertip dried blood spot (DBS) analysis offering a practical and cost-effective assessment method. This study analyses more than 590,000 globally sourced DBS samples, providing fatty acid profiles in whole blood across diverse populations. Results reveal significant global and demographic disparities in *n*-3 levels and *n*-6:*n*-3 ratios, with suboptimal *n*-3 levels and imbalanced *n*-6:*n*-3 ratios prevalent worldwide. While the findings align with general trends reported in previous global mapping studies on omega-3 status in red blood cells (RBC), they are based on whole blood measurements and highlight the utility of DBS testing for large-scale monitoring. These results underscore the need to enhance *n*-3 fatty acid intakes to help mitigate multiple global health challenges and demonstrate the utility of fingertip DBS testing as a practical approach to design dietary interventions to promote overall well-being.

Keywords Omega-3 fatty acids, Omega-6:omega-3 ratio, Biomarkers, Dried blood spot, Crowd science, Human biomonitoring, Public health surveillance, Large-scale population health

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Introduction

Alpha-linolenic acid (ALA, 18:3 *n*-3) and linoleic acid (LA, 18:2 *n*-6) are essential for human health [1], as they serve as precursors to the longer-chain polyunsaturated fatty acids (PUFAs) like arachidonic acid (AA, 20:4 *n*-6) and eicosapentaenoic acid (EPA, 20:5 *n*-3) [2]. The relative proportions of the *n*-6 and *n*-3 fatty acids in the body, captured in the *n*-6:*n*-3 ratio, are equally critical, as they determine the balance among proinflammatory and anti-inflammatory processes [3]. This balance influences the production of lipid mediators, such as the resolvins and protectins derived from *n*-3 fatty acids, which exhibit pro resolving properties, while *n*-6 derivatives including certain prostaglandins and leukotrienes, exert a proinflammatory role [4]. Understanding this interplay is essential for identifying dietary patterns and interventions that reduce disease risk and promote health.

The *n*-6:*n*-3 ratio also impacts the composition and functionality of cell membranes. At low dietary *n*-3 levels, the fatty acid intake has a significant influence on membrane composition. As *n*-3 levels increase beyond a certain threshold, membrane composition stabilizes, maintaining its integrity and functionality regardless of further dietary fluctuations [5]. This stabilization is crucial, as low dietary *n*-3 levels leave membranes more vulnerable to functional imbalances, which can strongly impact cell signalling, gene expression, and the production of lipid mediators [6–8]. When dietary fatty acid balance skews toward an elevated *n*-6:*n*-3 ratio, and the membrane *n*-3 levels are low, these processes promote a proinflammatory state that might exacerbate metabolic dysfunction [9–12].

Historically, diets rich in whole foods naturally provided a more balanced intake of *n*-6 and *n*-3 fatty acids. Modern dietary patterns have shifted toward an overconsumption of *n*-6-rich vegetable oils and processed foods, coupled with low intakes of *n*-3 fatty acids [11, 13]. This dietary shift has led to elevated *n*-6:*n*-3 ratios compared to the optimal range of 1–5:1 for health [14]. The resulting imbalance drives chronic low-grade inflammation, impairing cellular function, and contributing to the rising prevalence of metabolic dysfunction and inflammatory diseases [2, 15–17]. In particular, a higher *n*-6:*n*-3 ratio has been associated with increased inflammation and a heightened risk of autoimmune diseases, cancer, cardiovascular disease (CVD), diabetes, and other chronic conditions [18, 19].

Among *n*-3 fatty acids, the long-chain PUFAs EPA and docosahexaenoic acid (DHA, 22:6 *n*-3) play particularly important roles in maintaining cardiovascular health [20, 21], cognitive function [22–26], and modulation of inflammation [27], and are implicated in various neurological disorders [28, 29]. The impact of *n*-3 fatty acids depends not only on their absolute levels but also on their ability to counterbalance the high levels of *n*-6 fatty acids. These long-chain PUFAs influence the resolution of inflammation, the stabilization of neural membranes, and the modulation of oxidative stress [12, 30]. Importantly, studies assessing the effects of *n*-3 PUFAs must account for baseline blood levels and existing *n*-6:*n*-3 ratios, as these factors can strongly influence outcomes; Harris et al. showed that higher circulating *n*-3 PUFA levels correlate with a 15–18% reduction in all-cause mortality, particularly in individuals with initially low *n*-3 levels [15].

The literature on *n*-3 fatty acids, particularly EPA and DHA, is marked by notable discrepancies [15]. While many studies and meta-analyses report protective cardiovascular or overall mortality benefits, several well-known trials and observational cohorts have not reproduced these findings [31]. These inconsistencies arise from divergent study designs, differences in the methodologies used to assess *n*-3 fatty acid intake, and a frequent failure to account for baseline *n*-3 status or track improvements over time in intervention studies. Tools such as dietary surveys, are limited by recall bias and inaccuracies, highlighting the need for more objective measures [15, 31]. Analytical testing of biological samples offers direct insights into *n*-3 and *n*-6 fatty acid levels and consequently their potential physiological impacts; and is thus preferred for accurate evaluation of PUFA status [32, 33]. Importantly, it enables the determination of critical ratios, such as AA: EPA and total *n*-6:*n*-3, which are key indicators of fatty acid tissue balance and their physiological impacts [34], together with determination of *n*-3 levels (EPA + DHA), which reflects the biologically active omega-3 pool.

Dried blood spot (DBS) analysis has emerged as a practical non-invasive method for large-scale studies and global health monitoring [35]. DBS allows for minimally invasive sample collection while providing data that correlates well with red blood cell (RBC) measures like the omega-3 index (O3I) [36–39]. Moreover, DBS offers a cost-effective and scalable solution, enabling its application in large populations and across diverse geographical regions [35, 40, 41]. Whole-blood *n*-3 PUFA levels, measured using finger-prick DBS samples, include contributions from both plasma and RBCs and provide a comprehensive representation of fatty acid status [32]. This measure correlates with tissue levels and health outcomes such as CVD risk [40].

The present study leverages what is believed to be the largest global database of *n*-3 levels and fatty acid profiles from DBS analysis, encompassing more than 590,000 samples. As noted, among the various fatty acids, EPA and DHA are the most biologically and clinically relevant omega-3s, and their combined concentration in blood (EPA + DHA) is widely used as a practical and validated marker of omega-3 status. In addition, the AA:EPA ratio

is frequently used as an indicator of the balance between proinflammatory and anti-inflammatory lipid mediators, and the total *n*-6:*n*-3 ratio reflects broader dietary and metabolic PUFA patterns and is considered a marker of lipid balance at the populational level. Therefore, these three variables were selected for this study based on their established use in both clinical and epidemiological research, their functional relevance, and their interpretability in the context of nutritional surveillance and public health policy.

By mapping these levels across diverse regions and demographic groups, this study provides an unprecedented opportunity to evaluate geographic and demographic variations in fatty acid status. Additionally, the comparison of omega-3 supplement-users with non-users highlights the influence of supplementation on *n*-3 levels and fatty acid balance. By addressing both *n*-3 levels and *n*-6:*n*-3 imbalances, these findings have the potential to support public health strategies aimed at reducing the global burden of chronic diseases.

Materials and methods

Study design and data collection

This observational study employed a self-administered DBS fatty acid screening test analysed at the Vitas laboratory in Oslo, Norway. The home-based fingerstick collection method and test kits were distributed by Zinzino Operations (Sweden) through diverse channels, including health practitioners, therapists, web shops, and other sales outlets. By using DBS, broad participation is facilitated while minimizing the logistical challenges of traditional blood sampling, thereby enabling the collection of a large, international dataset.

Participant enrolment

Voluntary participants were recruited by filling in an online questionnaire, which gathered demographic data (age, sex, nationality, height, weight) and information on dietary supplement use. Those indicating use of omega-3 fatty acid supplements were asked to specify duration: "yes, for a little while", or "yes, for more than 120 days". Individuals who responded "no" or "yes, for a little while" were classified and stored in the dataset as "non-users of omega-3 supplements", whereas those responding "yes, for more than 120 days" were categorized as "users of omega-3 supplements". Details regarding the specific brands, dosages, or formulation of omega-3 products were not collected. The 120 day cut-off was chosen because it approximates the lifespan of RBCs [42]. Since the measures are based on whole blood, which includes RBCs, this approach ensures that reported supplement use reflects a sufficient duration to affect RBC fatty acid composition (i.e., long-term omega-3 incorporation). Although the questionnaire distinguished between short- and long-term supplement use, short-term users were not retained as a separate category in the historical dataset used for this analysis.

To address concerns regarding the study's generalizability and to avoid potentially inflated *n*-3 levels from participants using the test kit provider's own supplements, individuals who reported consuming omega-3 supplements from Zinzino Operations (Sweden) were excluded from all analyses. This step was taken to minimize bias arising from brand-related supplementation patterns.

Inclusion criteria

A total of 595,480 fatty acid profiles were included in this study. Inclusion criteria were having answered all questions in the online questionnaire. Participants who answered "other" for sex and/or reported ages > 119 years were not included in the study.

Data management

Participants were informed about how their data would be managed and protected. They were required to actively consent to having their personal health data analysed by clicking an "I agree" button at the end of the questionnaire to be included in the study. Participants were also given instructions on how they could withdraw from the study and have their data deleted by sending an email including their personal test ID. The data presented in this study were collected during the period from 1st of April 2012 to 27th of September 2023. The study protocol was approved by the Joint Research Ethics Committee at the University of Central Lancashire, United Kingdom (HEALTH 01195).

DBS collection

Fatty acid profiles were obtained from whole-blood using DBS. DBS is a bio-sampling method in which capillary blood, obtained by a finger-prick lancet, is captured in spots on DBS collection cards [43]. Participants received a pre-assembled sampling kit containing: (i) a single-use lancet, (ii) a Whatman 903 filter card, and (iii) and an aluminium-laminate pouch pre-loaded with calcium-chloride desiccant. After finger-prick collection, instructions were to allow the card to air-dry horizontally at room temperature for a minimum of 10 min before placing it into the aluminium bag and seal it for preservation until shipment to Vitas laboratory in Oslo, Norway for analysis. Samples were shipped at ambient temperature by regular post within the Nordic region or via consolidated express air transport (≤ 48 h) from hubs at remote locations. The feasibility and general stability of self-sampled DBS specimens sent by mail have been previously demonstrated [44].

Fatty acid analyses of DBS

Fatty acid profiles obtained from DBS samples were analysed using established methods at the Vitas laboratory [45]. Upon arrival at the laboratory, DBS samples were stored at 4 °C prior to analysis within one week. The DBS samples were methylated with 0.5 M sodium methoxide for 20 min at 50 °C to facilitate fatty acid methyl ester (FAME) formation. FAMEs were then extracted into hexane [46] and analysed by gas chromatography–flame ionization detection (Agilent Technologies, 8890) with a TR–FAME column (length, 30 m; internal diameter, 0.25 mm; and film thickness, 0.25 μm; Thermo–Fisher Scientific).

Eleven fatty acids were identified as the major components of whole blood and quantified using gas chromatography. Each FAME was identified by comparing retention time to the external FAME reference standard (USP – FAME standard mixture, Merk). Fatty acid values are expressed as the percentage area of the total identified FAMEs, calculated as the sum of the 11 quantified fatty acids. The measured and identified fatty acids were: palmitic acid (PA, 16:0), stearic acid (SA, 18:0), oleic acid (OA, 18:1 *n*-9), LA, γ-linolenic acid (GLA, 18:3 *n*-6), ALA, dihomo-γ-linolenic acid (DGLA, 20:3 *n*-6), AA, EPA, docosapentaenoic acid (DPA, 22:5 *n*-3), and DHA.

Whole blood *n*-3 fatty acid levels, herein referred to as "*n*-3 levels," were defined as the sum of EPA and DHA percentages, based on their established clinical relevance. The AA: EPA ratio was calculated using AA and EPA, and the total *n*-6:*n*-3 ratio was calculated as the sum of the measured *n*-6 fatty acids (LA, GLA, DGLA, AA) divided by the sum of the measured *n*-3 fatty acids (ALA, EPA, DPA, DHA).

Statistical analysis

A summary of the statistics is presented as mean or median values, as appropriate. Continuous data is expressed as mean ± standard deviation (SD) or standard error of mean (SEM), as specified, while categorical variables are expressed as proportions (%). Ratio data are expressed as median and the interquartile range (IQR) to account for potential skewness and ensure robust descriptive statistics. Confidence intervals (95% CI) are used for graphical representation of both mean and median values to illustrate variability and precision. Between-group differences were assessed using independent *t* tests or one-way ANOVA for unadjusted comparisons. For adjusted analyses, multiple linear regression models were used to examine the associations between omega-3 supplement use and *n*-3 levels, controlling for age, body mass index (BMI), and sex. Additionally, analysis of covariance (ANCOVA) was employed to compare *n*-3 levels across continents, with omega-3 supplement use and continent as fixed factors and age, BMI, and sex as covariates. Post-hoc comparisons were adjusted using Bonferroni correction. Effect sizes were calculated using Cohen's *d*, rank biserial correlation coefficients, or partial eta-square where appropriate. Significance was set to $P < 0.05$.

To ensure a representative dataset for country-level analysis, countries with fewer than 35 samples were excluded when presenting the mean *n*-3 levels, and median AA: EPA and total *n*-6:*n*-3 ratios in different countries. This decision was based on the Schuchardt et al. report [47], where the lowest number of participants included in any of their countries was 35, ensuring a reliable estimate of average levels. Additionally, colour-gradient maps were created to illustrate the global variations in *n*-3 levels and total *n*-6:*n*-3 ratio. For this visualization, countries with fewer than 35 samples were also excluded to minimize the impact of small sample sizes on the accuracy of the map representation. For continental group analysis, all countries were included regardless of their sample size to provide a comprehensive overview and to capture global trends. All statistical analyses were performed using JMP Pro (SAS Institute Inc., 1989–2019), IBM SPSS Statistics 28 and Microsoft Office Excel software. IBM SPSS Statistics 28 and Microsoft Office Excel were also used to create the figures.

Results

Demographic characteristics of the population

The mean age of the study participants was 47.3 ± 16.4 years. Only a small proportion of the participants were under the age of 20 (approximately 5%) or over the age of 80 (approximately 1.5%). Approximately 64% of the participants were females. Among adults over the age of 20 years, 48.5% were classified as "normal weight" based on their BMI. Although participants reported nationalities from all continents, 92.8% identified as European. Nationality was split into six regions, and European nationality was further split into four regions. A detailed description of the overall population demographics is presented in Table 1, while a breakdown by region, including sample distribution, omega-3 supplement use, and demographic characteristics, is shown in Table 2.

A general linear model (GLM) univariate analysis revealed statistically significant differences in omega-3 supplement use across continents ($F(6, 595582) = 1325.040$, $p < 0.001$, $\eta^2 = 0.001$). However, the effect size was extremely small, indicating minimal practical variation in supplementation prevalence across continents. Within Europe, regional differences in supplement use were also significant ($F(3, 552{,}913) = 2509.617$, $p < 0.001$, $\eta^2 = 0.013$), but the effect size remained small.

Table 1 Population demographics, lifestyle habits, geographical distribution, and omega-3 supplementation use

	n	% of total	Mean	SD
Age (years)	595,480		47.3	16.4
Weight (kg)	595,480		74.3	17.1
Height (cm)	595,480		170.2	11.8
BMI (kg/m^2 in those age > 20 years)	562,369		25.7	4.5
Sex				
Female	379,055		63.7	
Male	216,425		36.3	
Age group				
Children (< 10 years)	8635			
Adolescents (10–19 years)	24,476			
Young adults (20–39 years)	150,114			
Middle-aged adults (40–59 years)	271,105			
Old adults (60–79 years)	131,788			
Elderly (> 80 years)	9362			
BMI category (excluding age < 20)				
Underweight	11,445	2.0		
Normal weight	272,766	48.5		
Overweight	188,764	33.6		
Obese	89,394	15.9		
Continent				
Africa	2863	0.5		
Asia	14,875	2.5		
Europe	552,808	92.8		
North America	16,328	2.7		
Oceania	8330	1.4		
South America	276	0.1		
European region				
Central and Eastern Europe	138,499	25.1		
Northern Europe	199,427	36.1		
Southern Europe	16,915	3.1		
Western Europe	197,967	35.8		
Omega-3 supplement use				
No	519,304	87.2		
Yes	76,176	12.8		

Table 2 Regional demographics, geographical distribution, and omega-3 supplement use

Continent	*N*	% of Total	% Using Supplements	% Female	Mean Age
Total	595,589	100%	12.8%	63.7%	47
Africa	2863	0.5%	15.5%	60.8%	47
Asia	14,875	2.5%	7.8%	60.3%	49
North America	16,328	2.7%	16.5%	61.6%	49
Oceania	8330	1.4%	12.9%	66.3%	47
South America	276	0.05%	12.0%	61.6%	45
Europe	552,917	92.8%	12.8%	63.8%	47
European region					
Central and Eastern Europe	138,608	25.1%	8.2%	58.7%	46
Northern Europe	199,427	36.1%	17.6%	65.5%	46
Southern Europe	16,915	3.1%	7.6%	57.7%	49
Western Europe	197,967	35.8%	11.6%	66.0%	48

Table 3 Mean *n*-3 levels (EPA + DHA), and median AA: EPA and total *n*-6:*n*-3 ratios in whole blood in different continents and European subregions

Continent	Subregion	*n*-3 level (%)	AA: EPA ratio	Total *n*-6:*n*-3 ratio
		Mean (SD)	Median (IQR)	Median (IQR)
Africa		3.3 (1.4)	23.3 (24.9)	7.7 (3.7)
North America		2.8 (1.5)	21.2 (19.9)	8.8 (4.4)
South America		3.5 (1.4)	18.6 (19.2)	7.1 (3.4)
Asia		3.8 (1.6)	17.0 (20.6)	6.8 (3.4)
Oceania		3.7 (1.4)	12.5 (9.3)	6.2 (2.2)
Europe		3.6 (1.5)	13.2 (13.3)	6.3 (2.9)
	Central and Eastern Europe	3.0 (1.4)	21.9 (23.9)	8.0 (4.0)
	Southern Europe	3.4 (1.4)	21.5 (22.3)	7.6 (3.4)
	Western Europe	3.6 (1.4)	14.2 (11.9)	6.5 (2.5)
	Northern Europe	4.2 (1.5)	9.6 (7.3)	5.5 (1.9)

IQR, interquartile range

Global *n*-3 PUFA status

An analysis of *n*-3 levels, AA: EPA, and total *n*-6:*n*-3 ratios across different continents and regions – without distinguishing between omega-3 supplement users and non-users – revealed notable geographical variations (Table 3). The lowest mean *n*-3 levels were observed in North America and Africa. These continents also exhibited the highest median AA: EPA and total *n*-6:*n*-3 ratios. Asia, Oceania, and Europe exhibited the highest mean *n*-3 levels, and within Europe, Northern Europe displayed the highest mean *n*-3 levels. Oceania, Asia, and South America showed the lowest median ratios of total *n*-6:*n*-3. Additionally, the lowest median ratios of AA: EPA were observed in Oceania, Europe, and Asia, and within Europe, the lowest median AA: EPA ratio was seen in Northern Europe. Overall, these data demonstrate notable differences in key biomarkers within the different continents and within European subregions.

In addition to evaluating continental and regional variations, differences between the individual countries were investigated. The global distribution of *n*-3 levels, AA: EPA and total *n*-6:*n*-3 ratios in whole blood in different countries (≥ 35 samples) is presented by geographical regions in Tables 4, 5, 6, 7 and 8. These tables provide detailed insights into the variation of these metrics across different parts of the world, highlighting both regional trends and country-specific differences.

Table 4 Distribution of *n*-3 levels (EPA + DHA), AA: EPA ratio, and total *n*-6:*n*-3 ratio in whole blood in countries from Asia (≥ 35 samples)

Country	*n*	Age	*n*-3 level (%)	AA: EPA ratio	Total *n*-6:*n*-3 ratio
		Mean (SD)	Mean (SD)	Median (IQR)	Median (IQR)
Japan	69	46 (14.6)	5.1 (1.8)	7.8 (6.7)	4.8 (2.4)
Thailand	997	53 (13.3)	4.5 (1.6)	15.1 (18.3)	5.7 (2.5)
Philippines	565	49 (15.3)	4.2 (1.7)	14.8 (17.5)	5.9 (2.5)
Russia	1885	44 (16.8)	4.1 (1.6)	12.8 (17.5)	6.8 (3.4)
Taiwan	1439	53 (13.5)	4.1 (1.5)	14.9 (15.1)	6.6 (2.5)
Singapore	1294	55 (14.7)	4.1 (1.6)	17.7 (19.6)	6.3 (2.7)
Hong Kong	1802	51 (14.3)	4.0 (1.6)	12.7 (12.2)	6.3 (2.8)
Afghanistan	127	42 (20.0)	3.9 (1.7)	12.2 (11.0)	6.1 (2.8)
Malaysia	2228	50 (14.0)	3.9 (1.6)	21.3 (21.9)	6.6 (3.0)
Vietnam	43	47 (14.2)	3.9 (1.5)	20.0 (21.2)	6.6 (3.2)
Kazakhstan	114	42 (16.2)	3.9 (1.6)	17.5 (26.3)	7.6 (4.1)
Indonesia	54	47 (16.2)	3.8 (1.6)	20.0 (28.6)	6.9 (3.1)
United Arab Emirates	302	41 (13.1)	3.7 (1.5)	18.1 (18.8)	7.1 (3.3)
China	2299	49 (14.5)	3.3 (1.5)	19.4 (24.8)	7.9 (4.0)
Kyrgyzstan	75	50 (16.3)	3.3 (1.1)	25.9 (25.8)	8.1 (3.2)
Israel	117	48 (15.9)	3.1 (1.5)	20.9 (22.6)	8.6 (4.6)
Kuwait	39	44 (15.3)	3.0 (1.5)	29.7 (37.1)	9.2 (5.6)
India	1091	45 (15.0)	2.5 (1.5)	31.3 (42.7)	10.1 (6.9)

Table 5 Distribution of *n*-3 levels (EPA + DHA), AA: EPA ratio, and total *n*-6:*n*-3 ratio in whole blood in countries from Oceania (≥ 35 samples)

Country	*n*	Age	*n*-3 level (%)	AA: EPA ratio	Total *n*-6:*n*-3 ratio
		Mean (SD)	Mean (SD)	Median (IQR)	Median (IQR)
Australia	7940	47 (15.7)	3.7 (1.4)	12.5 (9.3)	6.2 (2.2)
New Zealand	361	47 (15.7)	3.4 (1.4)	12.2 (8.7)	6.5 (2.7)
Samoa	60	33 (24.2)	3.7 (2.1)	12.3 (12.1)	6.7 (4.4)

Table 7 Distribution of *n*-3 levels (EPA + DHA), AA: EPA ratio, and total *n*-6:*n*-3 ratio in whole blood in countries from North- and South America (≥ 35 samples)

Country	*n*	Age	*n*-3 level (%)	AA: EPA ratio	Total *n*-6:*n*-3 ratio
		Mean (SD)	Mean (SD)	Median (IQR)	Median (IQR)
North America					
Anguilla	51	39 (14.1)	3.7 (1.3)	13.2 (13.5)	5.8 (3.1)
Mexico	534	48 (14.4)	3.0 (1.4)	20.8 (21.8)	8.0 (3.8)
Canada	1600	50 (15.9)	2.9 (1.5)	15.1 (12.8)	7.6 (3.3)
United States	13,867	49 (16.4)	2.7 (1.5)	22.3 (20.5)	9.0 (4.5)
Guatemala	49	50 (15.8)	2.6 (1.0)	24.3 (18.8)	8.5 (3.8)
South America					
Brazil	80	40 (15.2)	3.7 (1.4)	18.8 (19.9)	6.4 (2.9)

Table 6 Distribution of *n*-3 levels, AA: EPA ratio, and total *n*-6:*n*-3 ratio in countries from Europe (≥ 35 samples)

Country	*n*	Age (mean)	*n*-3 level (mean)	AA: EPA ratio (median)	Total *n*-6:*n*-3 ratio (median)
Faroe Islands	1507	49 (17.2)	4.5 (1.7)	8.9 (7.2)	5.1 (2.1)
Norway	46,675	46 (16.7)	4.4 (1.7)	9.4 (8.1)	5.3 (2.1)
Belarus	60	45 (15.0)	4.4 (1.9)	11.9 (16.2)	6.1 (3.3)
Estonia	7158	42 (16.4)	4.2 (1.6)	10.2 (8.6)	5.5 (2.1)
Portugal	872	50 (15.7)	4.2 (1.4)	15.5 (16.4)	6.1 (2.5)
Finland	33,001	45 (16.1)	4.2 (1.4)	9.2 (6.2)	5.3 (1.7)
Lithuania	16,289	43 (17.1)	4.2 (1.6)	10.9 (10.5)	5.8 (2.4)
Iceland	1653	48 (16.2)	4.1 (1.8)	8.7 (6.7)	5.4 (2.2)
Latvia	13,770	44 (16.6)	4.1 (1.6)	10.6 (10.3)	5.9 (2.5)
Ukraine	1022	42 (15.1)	4.1 (1.6)	14.0 (18.9)	6.7 (3.5)
Sweden	79,374	48 (16.6)	4.0 (1.4)	9.6 (6.4)	5.5 (1.8)
Denmark	34,268	49 (16.3)	4.0 (1.6)	10.7 (9.0)	5.6 (2.2)
France	1764	50 (15.8)	4.0 (1.4)	13.2 (10.9)	6.1 (2.4)
Poland	11,868	47 (17.1)	3.9 (1.4)	13.2 (11.3)	6.0 (2.3)
Luxembourg	466	47 (16.0)	3.7 (1.4)	13.7 (11.9)	6.4 (2.5)
Albania	78	36 (21.2)	3.7 (1.9)	13.1 (15.3)	6.2 (2.8)
United Kingdom	12,761	44 (15.3)	3.7 (1.5)	13.9 (11.1)	6.3 (2.4)
Ireland	1243	43 (16.6)	3.6 (1.5)	13.9 (12.0)	6.4 (2.5)
Belgium	2432	48 (14.9)	3.6 (1.3)	14.3 (10.9)	6.5 (2.3)
Germany	77,401	50 (16.7)	3.6 (1.4)	14.3 (11.5)	6.5 (2.4)
Netherlands	26,399	47 (14.7)	3.6 (1.4)	15.1 (12.3)	6.7 (2.5)
Spain	5024	47 (14.8)	3.5 (1.4)	19.2 (20.6)	7.4 (3.4)
Moldova	85	45 (15.5)	3.4 (1.4)	20.0 (20.8)	7.8 (4.3)
Italy	5785	51 (15.3)	3.4 (1.3)	19.8 (18.4)	7.3 (3.0)
Malta	139	43 (16.1)	3.4 (1.3)	21.1 (18.9)	7.1 (3.5)
Bulgaria	829	40 (15.0)	3.3 (1.6)	25.0 (30.7)	8.6 (4.6)
Switzerland	16,682	50 (15.9)	3.3 (1.3)	16.4 (12.0)	7.1 (2.6)
Liechtenstein	165	48 (17.2)	3.3 (1.4)	16.7 (13.3)	7.3 (2.8)
Czech Republic	46,150	45 (16.4)	3.3 (1.3)	18.6 (14.4)	7.2 (2.5)
Austria	24,374	48 (16.4)	3.3 (1.3)	18.0 (13.9)	7.3 (2.6)
Slovenia	2592	47 (15.0)	3.2 (1.3)	22.3 (24.8)	8.2 (3.8)
Cyprus	1663	46 (15.3)	3.2 (1.2)	26.4 (26.0)	8.1 (3.5)
Slovakia	22,513	46 (16.4)	3.1 (1.3)	20.4 (17.9)	7.7 (2.9)
Greece	2676	50 (15.5)	3.0 (1.2)	29.0 (27.9)	8.5 (3.7)
Turkey	865	45 (14.9)	3.0 (1.4)	31.0 (48.5)	9.1 (4.8)
Croatia	550	46 (15.0)	3.0 (1.4)	31.5 (36.7)	9.2 (4.1)
Iran	41	41 (12.9)	2.9 (1.3)	23.7 (21.6)	8.3 (3.6)
Georgia	41	39 (14.9)	2.8 (1.1)	28.9	9.1
Montenegro	44	39 (16.6)	2.8 (1.4)	39.7 (44.8)	10.5 (5.4)
Romania	4467	46 (16.3)	2.8 (1.3)	30.4 (36.1)	9.5 (4.8)
Bosnia and Herzegovina	378	50 (15.7)	2.6 (1.1)	44.4 (42.7)	10.6 (4.4)
Macedonia	319	42 (20.2)	2.5 (1.2)	41.2 (57.6)	11.0 (6.1)
Serbia	799	47 (16.0)	2.5 (1.1)	45.6 (43.8)	11.0 (4.7)
Hungary	46,499	48 (16.5)	2.4 (1.2)	37.0 (38.5)	10.7 (4.8)

The highest mean *n*-3 levels were seen in Ghana (5.4 ± 2.0), followed by Japan (5.1 ± 1.8), Nigeria (4.6 ± 1.7), Faroe Islands (4.5 ± 1.7), Thailand (4.5 ± 1.6), Norway (4.4 ± 1.7), and Belarus (4.4 ± 1.9). Conversely, the

Table 8 Distribution of *n*-3 levels (EPA + DHA), AA: EPA ratio, and total *n*-6:*n*-3 ratio in whole blood in countries from Africa (≥ 35 samples)

Country	*n*	Age	*n*-3 level (%)	AA: EPA ratio	Total *n*-6:*n*-3 ratio
		Mean (SD)	Mean (SD)	Median (IQR)	Median (IQR)
Ghana	37	43 (17.0)	5.4 (2.0)	8.7 (8.5)	5.1 (2.0)
Nigeria	35	43 (18.0)	4.6 (1.7)	13.7 (13.2)	5.8 (2.4)
Algeria	38	33 (24.5)	3.7 (1.6)	13.9 (11.2)	5.8 (3.0)
Eswatini	38	50 (18.0)	3.3 (1.2)	19.0 (13.4)	7.1 (3.3)
South Africa	2266	48 (15.8)	3.2 (1.3)	25.8 (25.0)	8.0 (3.6)
Botswana	39	49 (13.2)	2.9 (1.4)	30.0 (32.3)	9.3 (4.4)

highest median AA: EPA ratios were seen in Serbia (45.6 ± 43.8), Bosnia and Herzegovina (44.4 ± 42.7), Macedonia (41.2 ± 57.6), Montenegro (39.7 ± 44.8), Hungary (37.0 ± 38.5), Croatia (31.5 ± 36.7), and India (31.3 ± 42.7). Furthermore, the highest median total *n*-6:*n*-3 ratios were seen in Serbia (11.0 ± 4.7), Macedonia (11.0 ± 6.1), Hungary (10.7 ± 4.8), Bosnia and Herzegovina (10.6 ± 4.4), Montenegro (10.5 ± 5.4), India (10.1 ± 6.9), and Romania (9.5 ± 4.8).

The colour-gradient map shown in Fig. 1 provides a visual representation of the mean *n*-3 levels in whole blood, ranging from 2.4 to 5.4%, from countries with ≥ 35 samples. Notably, regions such as Northern Europe, East Asia, and Oceania exhibit the highest *n*-3 levels (light blue), indicative of higher *n*-3 PUFA status, whereas countries in North America, South America, and parts of Africa predominantly display lower *n*-3 levels (dark blue), indicative of lower *n*-3 PUFA status.

Similarly, the colour-gradient map shown in Fig. 2 illustrates the median total *n*-6:*n*-3 ratio in whole blood across countries with ≥ 35 samples, ranging from 5:1 to 11:1. Regions such as North America, South Asia, and parts of Europe exhibit the highest median ratios (dark blue), reflecting a more pronounced imbalance favouring *n*-6 fatty acids. Conversely, countries in Northern Europe and parts of East Asia demonstrate lower median total *n*-6:*n*-3 ratios (light blue), indicative of a more balanced ratio.

Predictors of *n*-3 levels: multiple regression analysis

To explore the overall differences in *n*-3 levels in the study population, a multiple linear regression analysis was conducted, including supplement use, age, BMI, and sex as predictors (Table 9). *N*-3 levels were modelled as the outcome variable, with self-reported supplement use as the main predictor, adjusting for age, BMI, and sex.

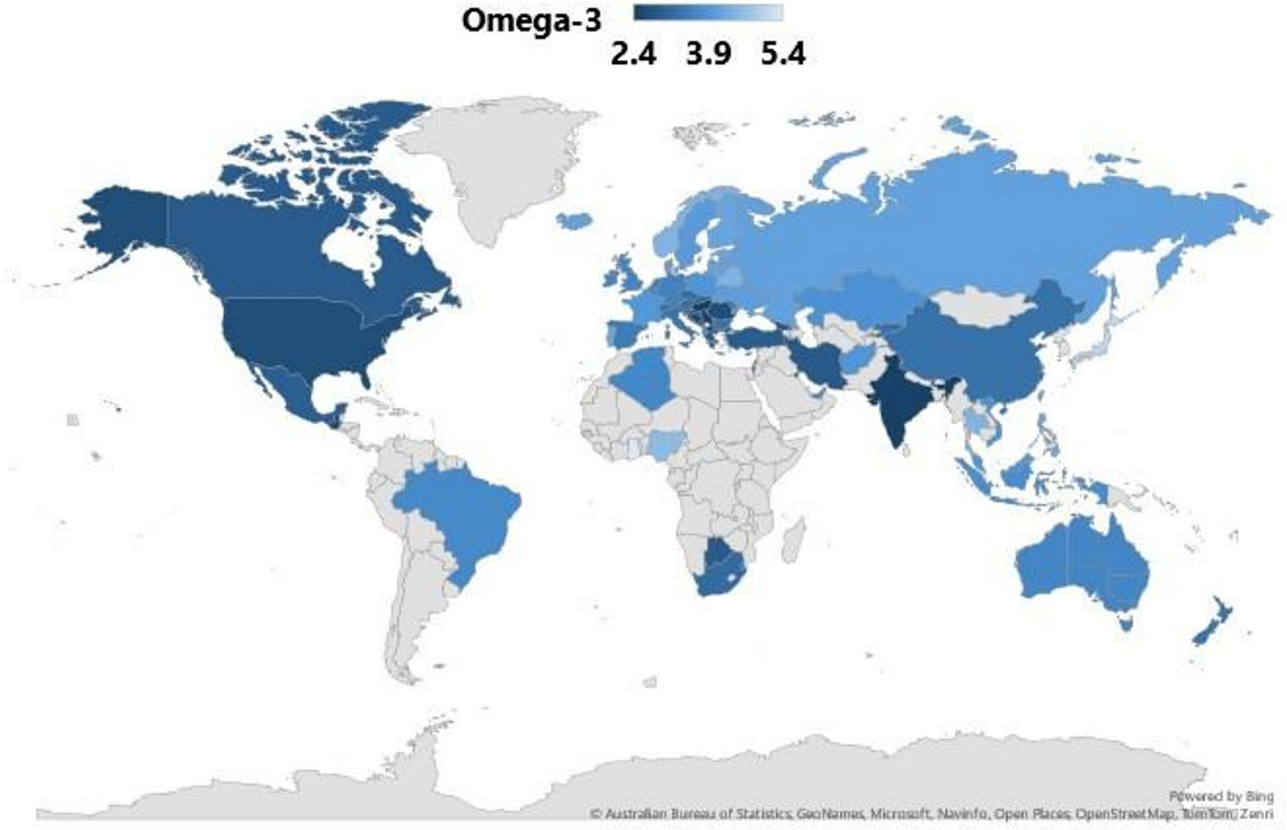

Fig. 1 Global distribution of *n*-3 levels (omega-3) in countries with ≥ 35 samples

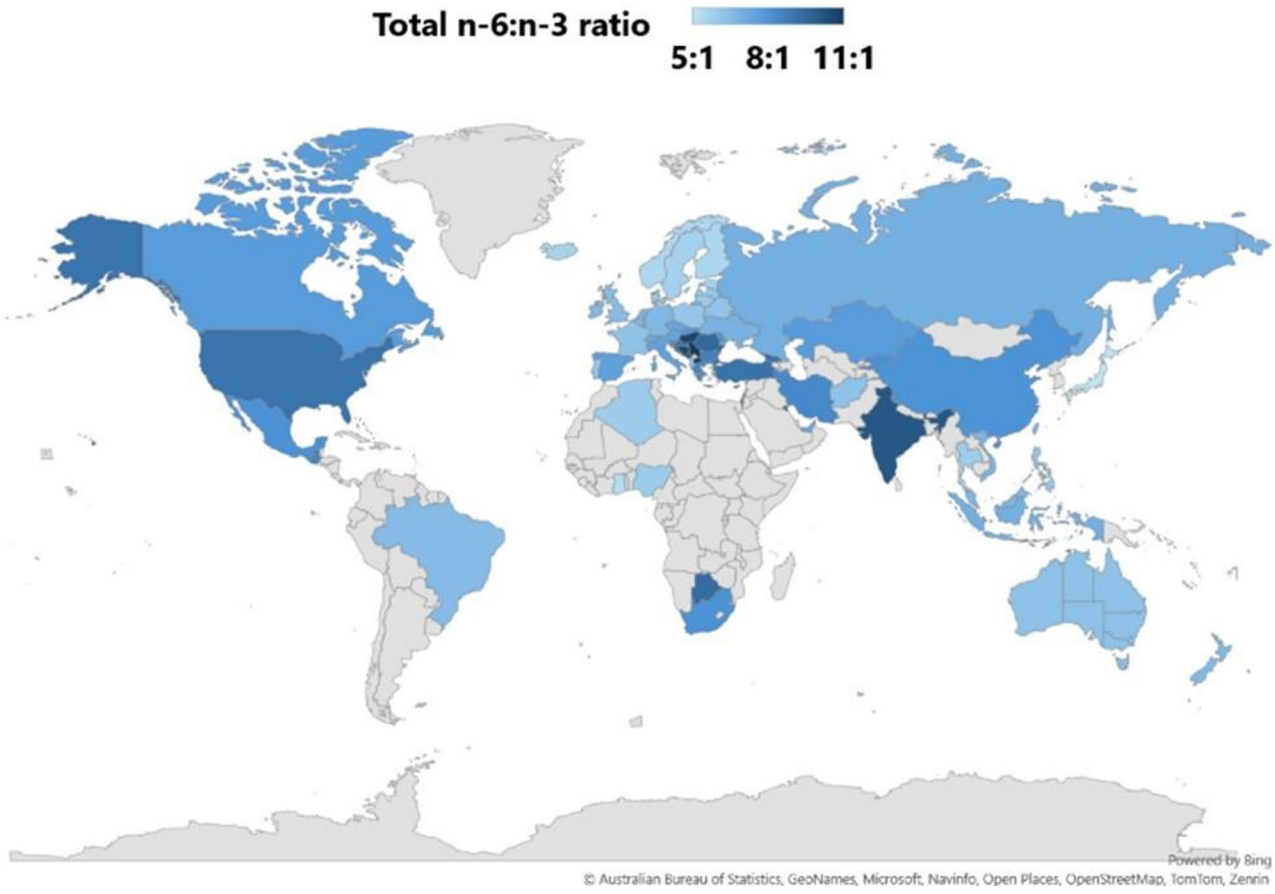

Fig. 2 Global distribution of total *n*-6:*n*-3 ratio in countries with ≥ 35 samples

Table 9 Multiple linear regression analysis predicting *n*-3 levels based on omega-3 supplement use, age, BMI, and sex

Variable	Unstandardized B (95% CI)	Standardized β	t-value	*p*-value
Supplement use	+ 1.008 (0.997, 1.020)	0.220	177.10	< 0.001
Age	+ 0.013 (0.012, 0.013)	0.137	105.46	< 0.001
BMI	-0.037 (-0.038, -0.036)	-0.111	-84.63	< 0.001
Sex	-0.188 (-0.196, -0.180)	-0.059	-46.79	< 0.001
(Constant)	3.092 (3.065, 3.118)	–	229.43	< 0.001

The regression model (R^2 = 0.083) showed that omega-3 supplement use was the strongest predictor (β = 0.220), followed by age (β = 0.137) and BMI (β = -0.111). Supplement users had 1.01% higher *n*-3 levels compared to non-users (95% CI: 0.997–1.020). Age and BMI explained less variation, while gender had minimal impact (β = -0.059).

Regional differences in *n*-3 levels by supplemental group

Among the 595,480 samples, the ANCOVA revealed significant continental differences in adjusted mean *n*-3 levels for both supplement users (F = 880.8, $P < 0.001$) and non-users (F = 642.3, $P < 0.001$), after controlling for age, BMI, and sex. (Fig. 3). Post-hoc comparisons with Bonferroni correction revealed specific regional patterns: North America consistently showed significantly lower *n*-3 levels regardless of supplement use. Notably, Europe and Oceania showed no significant differences in either group ($P = 1.000$ for both), while Asia-Europe comparisons were significant among non-users ($P < 0.001$) but not among supplement users ($P = 1.000$). Asia also differed significantly from South America among non-users ($P = 0.039$). The partial eta-squared values indicated that continent explained 0.7% of variance in *n*-3 levels (partial η^2 = 0.007, $P < 0.001$), while supplement use showed no significant main effect (partial η^2 = 0.000, $P = 0.001$). The continent × supplement interaction was statistically significant but with negligible effect size (partial η^2 = 0.000, $P = 0.001$). Covariates collectively accounted for 9.3% of variance (adjusted R^2 = 0.093), indicating that demographic factors substantially influence *n*-3 levels beyond geographic and supplementation effects.

Sex related differences in *n*-3 levels, AA: EPA, and total *n*-6:*n*-3 ratios

Next, sex-related differences in *n*-3 levels, AA: EPA ratio, and total *n*-6:*n*-3 ratio were examined. The analysis

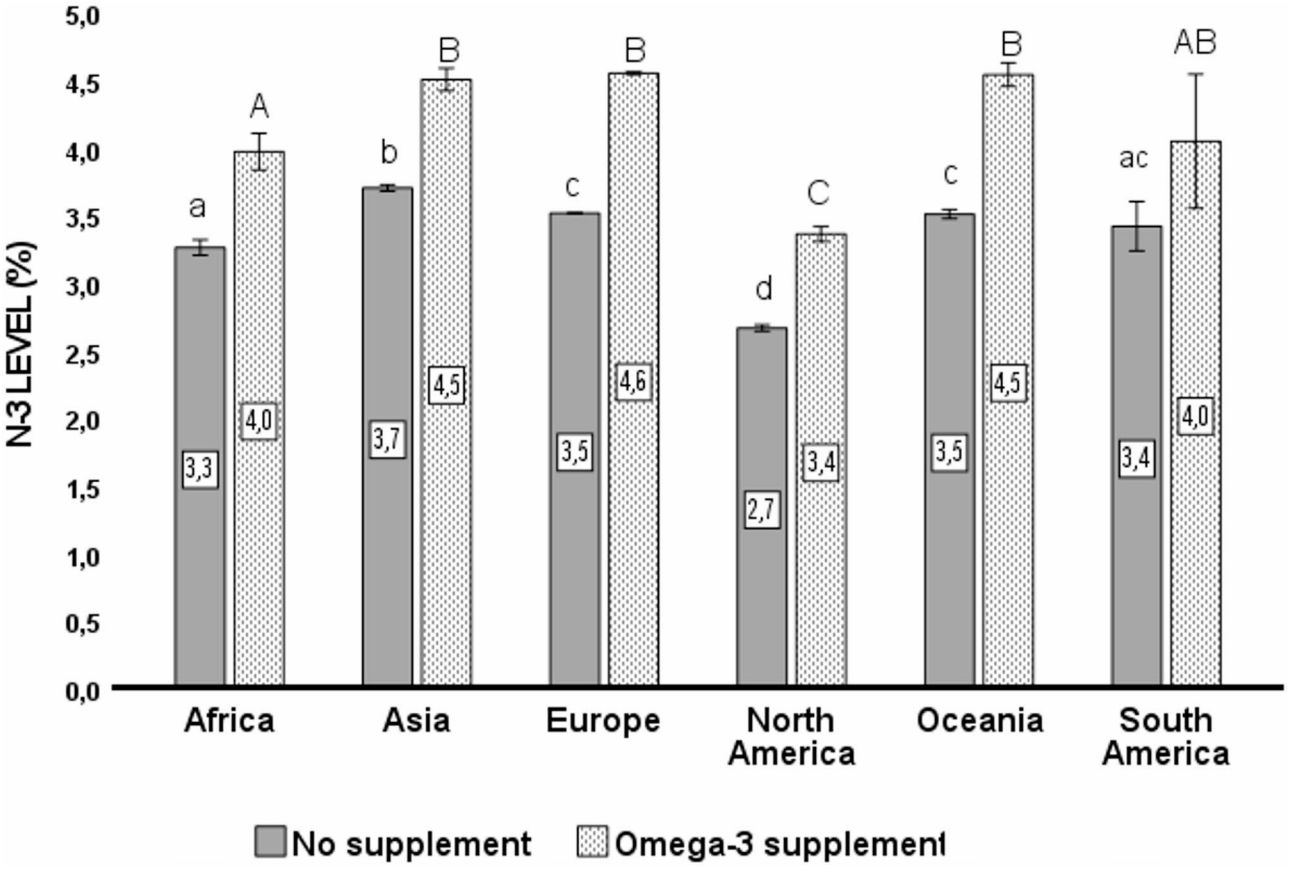

Fig. 3 Adjusted mean (± 95% CI) *n*-3 levels (EPA + DHA) by continent, stratified by omega-3 supplement use. Values represent estimated marginal means from analysis of covariance (ANCOVA), adjusted for age, BMI, and sex. Error bars indicate 95% confidence intervals. Lowercase letters (**a**, **b**, **c**, **d**) denote significant differences among continents within the non-supplement group, while uppercase letters (**A**, **B**, **C**) indicate significant differences among continents within the omega-3 supplement group. Bars sharing a letter are not significantly different (Bonferroni-corrected post-hoc test, $P < 0.05$)

Table 10 Differences in mean *n*-3 levels (EPA + DHA), median AA: EPA, and total *n*-6:*n*-3 ratios in whole blood between sexes

	Sex			
	Female	**Male**		
	Mean (SD)	**Mean (SD)**	***P*-value**	**Cohen's d value**
n-3 level	3.73 (1.52)	3.45 (1.53)	< 0.0001	0.184
	Median (IQR)	Median (IQR)	*P*-value	Rank-biserial correlation *r*
AA: EPA ratio	13.0 (13.2)	14.3 (14.5)	< 0.001	-0.055
Total *n*-6:*n*-3 ratio	6.4 (2.9)	6.4 (3.0)	< 0.001	-0.009

T-tests were used for comparison of means and Mann-Whitney U tests for comparison of medians. Effect sizes are reported as Cohen's d and rank-biserial correlation r, respectively.

revealed a statistically significant difference in mean *n*-3 levels between females and males, albeit with a small effect size (Cohen's $d = 0.184$, Table 10). Moreover, females had a slightly lower median AA: EPA ratio than males, whereas the median total *n*-6:*n*-3 ratio was approximately the same. The rank-biserial correlations were negligible ($r = 0.055$ and $r = 0.001$), suggesting minimal differences between groups (Table 10).

Variations in *n*-3 levels and total *n*-6:*n*-3 ratios across age groups and BMI categories in omega-3-supplement users vs. non-users

Exploratory analyses were also conducted to assess how *n*-3 levels and total *n*-6:*n*-3 ratios varied across age groups and BMI categories, stratified by omega-3 supplement use. A significant increase in *n*-3 levels was seen among the different age groups ($P < 0.0001$) and increased with age, with a corresponding decrease in total *n*-6:*n*-3 ratio with age (Fig. 4A). Omega-3 supplement-users had on average, higher *n*-3 levels than non-supplement users, and 1.56% and 1.20%, respectively, of the variance in *n*-3 levels among them could be attributed to their age group according to eta-squared values from the analysis. Furthermore, non-supplement users had higher median total *n*-6:*n*-3 ratios than supplement users (Fig. 4B).

The relationship between *n*-3 levels and BMI (ages > 20 and < 80 years) was also examined using one-way ANOVA. The results revealed a significant effect of BMI

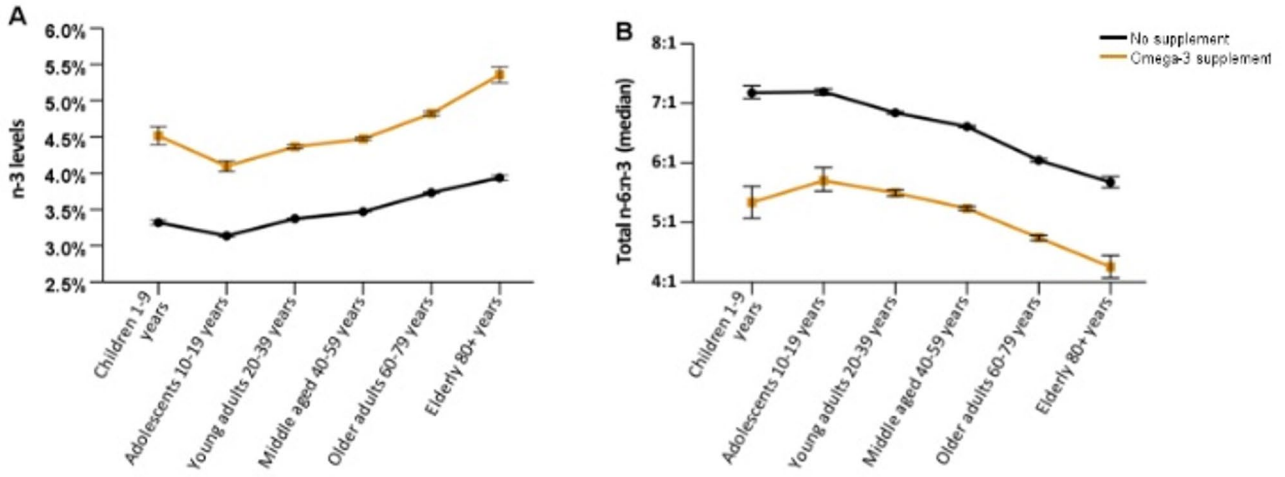

Fig. 4 Mean *n*-3 levels (EPA + DHA) and median total *n*-6:*n*-3 ratio across age groups, stratified by omega-3 supplementation status. (**A**) Mean *n*-3 levels (± 95% CI). (**B**) Median total *n*-6:*n*-3 ratio (± 95% CI)

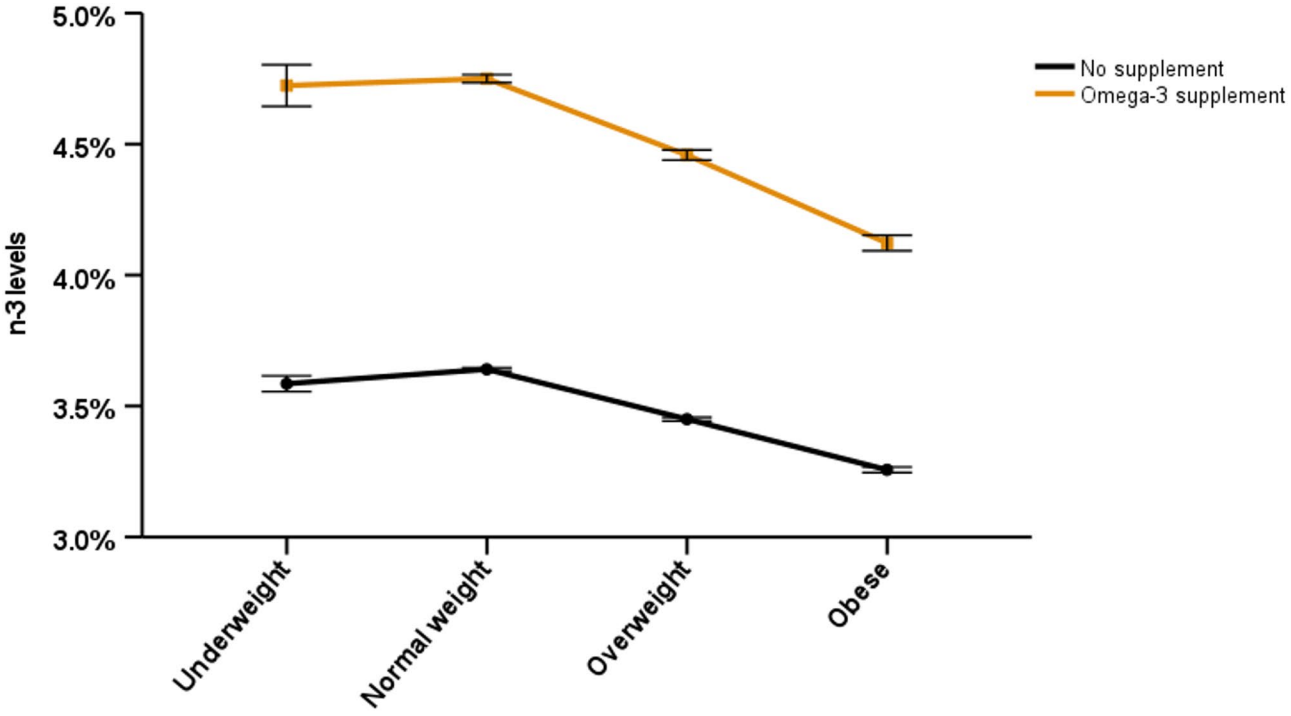

Fig. 5 Mean *n*-3 levels (EPA + DHA) in whole blood across BMI categories for omega-3 supplement-users and no supplement users. Error bars in figure represent ± 95% CI

on *n*-3 levels among both omega-3 supplement-users and non-users ($P < 0.0001$ for both). Eta-squared values indicated that BMI accounted for 1.6% and 0.95% of the variance in *n*-3 levels for omega-3 supplement-users and non-users, respectively (Fig. 5). Overweight and obese individuals had slightly lower mean *n*-3 levels compared to normal-weight individuals. Among omega-3 supplement-users, mean *n*-3 levels were 4.4% for overweight individuals and 4.1% for obese individuals, compared to 4.7% for those with normal weight. Similarly, among non-supplement users, mean *n*-3 levels were 3.4% for overweight individuals and 3.3% for obese individuals, compared to 3.6% for normal-weight individuals. Across all weight categories, omega-3 supplement-users consistently exhibited higher mean n-3 levels than non-users.

Discussion

In this study, an extensive investigation of global *n*-3 PUFA status and fatty acid imbalances was conducted by analysing more than 590,000 DBS samples from all

continents. The analysis revealed notable variations in mean *n*-3 levels, with significant continental differences observed among both omega-3 supplement and non-supplement users, adjusting for age, BMI, and sex. Individuals from Asia, Europe, and Oceania exhibited the highest adjusted mean *n*-3 levels, followed by those from South America and Africa, while the lowest levels were observed in North America. These findings are consistent with previous reviews on global omega-3 fatty acid blood levels [47, 48]. Moreover, individuals using omega-3 supplements had significantly higher *n*-3 levels compared to non-users, even after adjusting for age, BMI, and sex. This association supports the positive contribution of omega-3 supplementation to *n*-3 PUFA status, indicating that prolonged supplementation is associated with improved *n*-3 PUFA status [49, 50].

Furthermore, female sex and increasing age were associated with higher *n*-3 levels, while overweight and obesity were associated with lower *n*-3 levels. This aligns with existing evidence that obesity promotes systemic inflammation and cellular senescence, both of which are strongly linked to an imbalanced *n*-6:*n*-3 ratio [16].

A balanced dietary intake ratio of omega-6 and omega-3 fatty acids is considered crucial for maintaining overall health [51–53]. A dietary intake ratio of 1–5:1, typically referring to LA and ALA, is considered optimal [14], yet several studies report in take ratios in almost all populations ranging from 10–25:1 [10, 18, 54]. Biomarker levels measured in blood provide a different perspective, reflecting the combined effects of dietary intake, metabolism, and tissue incorporation. Previous studies on whole blood have reported *n*-6:*n*-3 ratios ranging from 6:1 to 7.5:1 in healthy, non-supplemented populations and lower ratios (~3:1 to 4:1) with omega-3 supplementation [55]. In this study, the total *n*-6:*n*-3 ratio ranged from 6.2:1 to 8.8:1 when analysed at the continental level. These values are consistent with previous whole blood findings and highlight the importance of considering the blood pool (e.g., DBS, RBC, platelet, or plasma phospholipids) and analytical technique (e.g., extraction and fractionation protocols) when interpreting fatty acid ratios. For instance, RBC-based assays measure membrane-incorporated fatty acids at a steady state reflecting ~120 days of turnover, whereas DBS captures both RBCs and plasma compartments, potentially yielding different *n*-6:*n*-3 ratios despite measuring the same underlying fatty acid classes. While this study cohort may reflect a more health-conscious subset of the population – given their participation in home-based health testing – the results indicate that total *n*-6:*n*-3 ratios in whole blood remain above levels typically associated with omega-3 supplementation. These ratios, ranging from 6.2:1 to 8.8:1 across continents, reflect broader dietary patterns and suggest that even health-conscious individuals may not achieve optimal fatty acid balance without targeted dietary interventions. The highest median total *n*-6:*n*-3 ratio was observed among participants from North America, reflecting dietary patterns characterized by a high intake of *n*-6 rich seed oils, processed foods, and insufficient consumption of *n*-3 rich foods or omega-3 supplements [51]. None of the continental groups displayed a median ratio below 5:1; within Europe, subregions such as Northern Europe exhibited the lowest total *n*-6:*n*-3 ratios, consistent with dietary patterns in Nordic countries [54].

Elevated *n*-6:*n*-3 ratios are broadly associated with metabolic dysfunction due to their role in promoting proinflammatory lipid mediators [3, 16, 18, 19, 56, 57]. Such imbalances have been linked to a range of chronic conditions, including CVD [10], cancer [19, 58], osteoarthritis [16], metabolic dysfunction [56], and autoimmune disorders [59]. These proinflammatory states are particularly prevalent in Western societies, where they coincide with a higher incidence of conditions like CVD, asthma, and cancer [14]. The AA: EPA ratio is considered a more specific marker of inflammatory balance, reflecting the interplay between AA and EPA [60]. AA initiates inflammation by facilitating the production of proinflammatory eicosanoids and cytokines, while EPA promotes its resolution by generating resolvins, which resolve inflammation and promote tissue regeneration [61]. The median AA: EPA ratio at a continental level was highest among African individuals, with a median of 23.3, and lowest in Oceanian individuals, with a median of 12.5. Africa exhibited a unique fatty acid profile, with moderately higher *n*-3 levels than North America, but the most unfavourable median AA: EPA ratio observed in this study. The elevated AA: EPA ratio in Africa is likely influenced by genetic predispositions (*FADS* variants) and dietary patterns with higher omega-6 intake [62]. When comparing European subregions, the lowest AA: EPA ratio was seen among Northern Europeans, with a median of 9.6. Notably, Takahasi et al. [63]. reported an increased prevalence of complex coronary lesions in patients with low plasma EPA: AA ratios. In general, a lower EPA: AA ratio is associated with greater risk of CVD, especially coronary heart disease (CHD) [21, 64]. When the data were analysed as an EPA: AA ratio (the inverse of the AA: EPA ratio), the results revealed a globally low value, consistent with the high AA: EPA ratios observed across regions. These findings align with evidence supporting the sensitivity of whole blood DBS markers in capturing regional variations influenced by dietary and genetic factors.

Findings from the Lyon Diet Heart Study provide evidence that a total *n*-6:*n*-3 ratio of 4:1 is associated with a 70% decrease in mortality in the secondary prevention of CVD [65]. In the context of the Lyon Diet Heart Study, in addition to the beneficial health outcomes associated

with a more balanced *n*-6:*n*-3 ratio, blood levels of EPA and DHA (*n*-3 levels) have been shown to be related to several beneficial health outcomes, including cardiovascular health [66]. Furthermore, specific ratios have displayed distinct effects in various health contexts. For instance, an omega-6 to omega-3 ratio of 2.5:1 has been shown to reduce rectal cell proliferation in patients with colorectal cancer [65], while a ratio of 5:1 has demonstrated beneficial effects in asthma patients [57]. Additionally, evidence suggests that a balanced *n*-6:*n*-3 fatty acid profile could mitigate the risk of breast cancer [67], and a high dietary ratio of *n*-6:*n*-3 fatty acids has been shown to correlate with higher prostate cancer risk among white men [68]. Overall, the results highlight total *n*-6:*n*-3 ratios exceeding the presumed optimal range, suggesting a potential need to address these imbalances on a global scale to mitigate the risk of proinflammatory states and its associated diseases.

Several factors may underlie the observed geographical differences, of which dietary patterns may play a pivotal role. Common to the countries with lower mean *n*-3 levels is that they often have diets with limited amounts of omega-3 fatty acids and higher consumption of processed and ultra-processed foods rich in omega-6 fatty acids, whereas the contrary is seen in the countries who displayed higher mean *n*-3 levels. The distribution of *n*-3 levels thus underscores the significant impact of regional dietary habits on *n*-3 PUFA status across diverse populations. Regions with diets traditionally rich in fish, such as many parts of Asia, Oceania, and Europe [69], tend to exhibit higher *n*-3 PUFA intake and consequently have higher *n*-3 levels. Conversely, North American dietary habits are characterized by higher consumption of processed and ultra-processed foods [70], and lower intake of fish [71], which contributes to the lower *n*-3 levels observed in this population and the higher AA: EPA and total *n*-6:*n*-3 ratios. These findings align with those of Naylor et al. [69], who reported varying levels of fish consumption across continents, with Asia, Europe, and Oceania exceeding the global average, while Africa and South America fall below, and North America just below. While the present study did not measure fish consumption, it is important to note that blood levels of EPA and DHA, which were analysed in this dataset, have previously been shown to be highly correlated with self-reported fish consumption [72] and the use of omega-3 supplementation [73].

Omega-3 supplementation was identified as a significant and independent contributor to *n*-3 PUFA status across diverse populations, consistent with previous studies [72]. However, it is important to note that other dietary influences were not measured in this study. The adjusted difference in *n*-3 levels between omega-3 supplement-users and non-users was modest but statistically robust, aligning with prior findings that such small increases in *n*-3 levels may have limited impact on major health outcomes [74]. This suggests that while omega-3 supplementation does influence *n*-3 PUFA status, achieving meaningful physiological and clinical benefits may require more substantial improvements in *n*-3 levels. While regional differences in omega-3 supplement use may contribute to continental disparities in *n*-3 PUFA status, ANCOVA results indicated that supplement use explained a modest proportion of variance (partial η^2 = 0.05) compared to the combined influence of demographic covariates. Nevertheless, in some regions or countries, particularly those with lower baseline levels, supplement use may still represent an important tool for improving *n*-3 PUFA status.

Furthermore, the analysis indicated that sex, BMI, and age were all independently associated with *n*-3 levels and the total *n*-6:*n*-3 ratio, albeit with a small effect size. Consistent with the literature, the data demonstrate that female sex and increasing age are positively associated with *n*-3 PUFA status [75, 76]. While omega-3 supplementation had consistent impact across all continents, its role must be considered alongside dietary intake patterns rich in marine sources of *n*-3 PUFAs to effectively promote optimal *n*-3 PUFA status and more balanced *n*-6:*n*-3 ratios. The lack of knowledge regarding type of omega-3 supplements used, their dosage, or type of stabilizing agent is a constraint of this study and could explain why optimal levels were not always achieved. This highlights the importance of further investigating the specific characteristics of omega-3 supplementation, including dosage, formulation, and duration, to better understand its role in improving *n*-3 PUFA status.

In an era of rising proinflammatory states, addressing global fatty acid profiles is increasingly important for tackling health associated challenges. The present study highlights the positive association between omega-3 supplementation and improvements in *n*-3 PUFA status, reinforcing the potential benefits of proactive dietary choices in achieving optimal health. In addition, this analysis provides unique whole blood estimations of *n*-3 PUFA status, expanding the geographical scope to include countries previously not assessed in RBC-based global studies. By incorporating these regions, particularly within Europe, this work complements previous research and offers a more comprehensive understanding of *n*-3 PUFA status trends and fatty acid balance. Although most samples originated from Europe, considerable numbers (42,672 samples) were also included from other continents, allowing for broader exploratory comparisons. Nevertheless, the smaller sample sizes from certain regions might necessitate somewhat more cautious interpretation of regional findings.

While direct comparisons with RBC-based studies are not feasible due to differences in the biological compartments measured, the global trends observed in the present study aligns with prior findings. This consistency supports the interpretation of relative differences, such as identifying regions with lower or higher *n*-3 levels, even though absolute values from these two measurement methods cannot be directly equated. This study also considers key ratios – AA: EPA and total *n*-6:*n*-3 – that provide valuable context for interpreting potential inflammatory balance and dietary fat quality, respectively. The results underscore the beneficial impact of omega-3 supplementation as a modifiable factor for improving fatty acid status. However, the forms of supplement consumed, which may influence bioavailability [77], and various other factors, were not accounted for in this study. As such, the findings represent a general overview of global trends in *n*-3 PUFA status, with potential variations depending on the specific forms of supplementation used. By identifying disparities in *n*-3 PUFA status, AA: EPA and total *n*-6:*n*-3 ratios across geographical regions and demographic groups, the study highlights opportunities for targeted dietary or public health interventions to address these imbalances and promote better health outcomes.

Strengths and limitations

A major strength of the study lies in the extensive dataset. Unlike previous assessments of global *n*-3 PUFA status, which have employed varied methodologies and reported fatty acid data across different blood fractions, the current study ensured methodological uniformity. This standardization not only enhances the reliability of findings across different populations but also strengthens the overall validity of the findings. However, some limitations should be considered. A complete global map of blood levels of *n*-3 PUFAs could not be provided due to the exclusion of numerous countries due to insufficient samples. Reliance on self-reported population demographics represents another limitation, as self-selection bias may have influenced the dataset, potentially leading to underrepresentation of individuals from underdeveloped countries or lower socioeconomic backgrounds.

While home collection and ambient shipping may introduce some random variability in PUFA measurements, such variability is unlikely to systematically bias the results because random errors tend to cancel out with large sample sizes, strengthening the robustness of the findings. Moreover, the risk of degradation is mitigated through rapid transport and optimized packaging procedures, including sealed aluminium-laminate pouches with calcium chloride desiccant. These measures help preserve PUFA integrity and support the assessment of relative trends under standardized conditions [78]. A further limitation is the potential inclusion of repeat testers. Due to data anonymization and the nature of the home test kit system, it was not possible to reliably identify and exclude individuals who may have submitted multiple samples. Nevertheless, as the majority of users only test once, it is expected to introduce minimal bias. In addition, although the questionnaire distinguished between short- and long-term supplement use, individuals reporting short-term use (< 120 days) were grouped with non-users in the dataset. While the 120-day threshold aligns with the lifespan of RBCs and was selected to reflect a biologically meaningful exposure period, existing evidence suggests that even shorter durations of supplementation may lead to measurable increases in omega-3 status [79]. This classification approach may therefore have introduced misclassification bias, potentially attenuating observed differences between users and non-users. Specifically, individuals who had recently initiated supplementation but were grouped with non-users may have already begun to exhibit elevated *n*-3 levels, thereby reducing the contrast between the two groups. Future studies with access to detailed supplementation duration data could enable more nuanced modelling of time-dependent effects through continuous or tiered exposure variables.

Conclusions

In conclusion, the present study, involving more than 590,000 DBS samples from diverse populations worldwide, underscores the value of DBS analysis as a powerful tool for assessing global trends in *n*-3 PUFA status and imbalances in *n*-6:*n*-3 ratios. The findings reveal significant geographical and demographic variations in *n*-3 levels and *n*-6:*n*-3 ratios, demonstrating the potential of this technique for large-scale health monitoring and nutritional epidemiology. Key insights include the identification of regional disparities and a consistent, statistically significant association between omega-3 supplement use and improved *n*-3 PUFA status globally (+ 1.0% mean difference), although this effect was insufficient to offset regional dietary imbalances.

The widespread prevalence of inadequate *n*-3 levels and unbalanced *n*-6:*n*-3 ratios highlight a pressing global nutritional challenge, emphasizing the critical need for public health initiatives aimed at increasing *n*-3 PUFA intake through both dietary sources and supplementation. By leveraging DBS analysis, the current study provides a robust foundation for future research to explore the determinants and consequences of fatty acid imbalances and their relationship to health outcomes.

Abbreviations

ALA	Alpha-linolenic acid
AA	Arachidonic acid
BMI	Body mass index

CVD	Cardiovascular disease
CHD	Coronary heart disease
DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DBS	Dried blood spots
EPA	Eicosapentaenoic acid
FADS	Fatty acid desaturates
FAME	Fatty acid methyl ester
IQR	Interquartile range
LA	Linoleic acid
OA	Oleic acid
n-3	Omega-3
O3I	Omega-3 index
n-6	Omega-6
PA	Palmitic acid
PUFAs	Polyunsaturated fatty acids
RBC	Red blood cells
SD	Standard deviation
SEM	Standard error of mean
SA	Stearic acid
GLA	γ-linolenic acid

Author contributions
O.E. conceptualized the study. Methodology was developed by O.E., T.G., T.B., M.T., and E.G. M.T. and E.G. were responsible for software development and validation. Formal analysis was conducted by M.T. and E.G., with M.T. leading the investigation. Data curation was handled by M.T. and E.G. The original draft of the manuscript was prepared by M.T., while M.T., E.G., A.M.R., P.C. and C.R. contributed to reviewing and editing the manuscript. Visualization was performed by M.T. and E.G. Project administration was overseen by C.R. All authors reviewed and approved the final manuscript.

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Data availability
The data sets generated and/or analyzed during the current study are not publicly available due to proprietary data restrictions but are available from the corresponding author upon reasonable request. Requests for data access should include a clear explanation of the purpose and scope of the intended use and may require additional ethical approval depending on the request.

Declarations

Ethics approval and consent to participate
The study protocol was approved by the Joint Research Ethics Committee at the University of Central Lancashire, United Kingdom (Reference Number: HEALTH 01195). Participants were informed about how their data would be managed and protected, and they actively consented to having their personal health data analyzed by clicking an "I agree" button at the end of the questionnaire. Participants were provided with clear instructions on how to withdraw from the study and have their data deleted by sending an email including their personal test ID.

Consent for publication
Not applicable.

Competing interests
M.T. is affiliated with Bioactive Foods AS, a company owned by Zinzino Operations AB. C.R. and E.G. are directly affiliated with Zinzino Operations AB. T.G. is the CEO and co-founder of Vitas Analytical Services and serves as a member of Zinzino's scientific advisory board. T.B. is affiliated with Vitas Analytical Services. P.C., and A.M.R. are consultants on Zinzino's Scientific Advisory Board. While these affiliations may represent potential competing interests, the study was conducted objectively, and the data analysis and interpretation were performed independently of any commercial influence.

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