

RESEARCH ARTICLE

Total Omega-3 Fatty Acid, Eicosapentaenoic Acid (EPA), and Docosahexaenoic Acid (DHA) are Positively Associated with Sleep Duration in Children Aged 3–5 Years: A Preliminary Study

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Received date: Mar 21, 2026; Revised date: May 4, 2026; Accepted date: May 6, 2026

Abstract

BACKGROUND: Sleep is essential for children's growth and development, yet sleep disturbances remain common among preschoolers. Insufficient sleep has been linked to cognitive impairments, behavioral problems, and increased risk of obesity. Nutritional factors, particularly omega-3 fatty acids, may play a role in sleep regulation. Although previous studies have suggested that Eicosapentaenoic Acid (EPA) and Docosahexaenoic acid (DHA) are associated with improved sleep outcomes, yet data on omega-3 status and its relationship with sleep duration among Indonesian preschoolers remain scarce. Therefore, this study was conducted to analyse the association between Omega-3 fatty acids levels and sleep duration among preschool children in Indonesia.

METHODS: A cross-sectional survey was conducted among 72 children aged 3–5 years old. Sleep duration and disturbances of subjects were assessed using the Children's Sleep Habits Questionnaire–Abbreviated (CSHQ-A). Omega-3 fatty acids levels, including α -linolenic acid (ALA), EPA, and DHA, were measured using gas chromatography.

RESULTS: The median sleep duration was 11 hours (range 9–13.5 hours). Based on CSHQ-A scores, 93.1% of subjects experienced sleep disturbances. Mean total omega-3 fatty acid concentration was $225.47 \pm 80.9 \mu\text{mol/L}$. Significant positive correlations were observed between sleep duration and total omega-3 fatty acid ($r=0.298$; $p=0.011$), EPA ($r=0.233$; $p=0.049$), and DHA ($r=0.260$; $p=0.028$).

CONCLUSION: Omega-3 fatty acid levels, particularly EPA and DHA, were positively associated with sleep duration in preschool children. These findings provide preliminary evidence of the role of omega-3 in sleep regulation and underscore the importance of considering nutritional factors in efforts to improve sleep quality during early childhood.

KEYWORDS: omega-3 fatty acids, DHA, EPA, ALA, sleep duration, preschool children

Indones Biomed J. 2026; 18(3): 234-42

Introduction

Sleep is an essential neurophysiological process supporting children's physical growth, neurocognitive function, emotional regulation, and behavioral development.(1)

Children aged 3–5 years old are in a period of rapid growth and development.(2) Adequate sleep is particularly critical during these formative years to support optimal physical and neurocognitive outcomes. Adequate sleep restores energy, enhances cognition, strengthens immunity, and consolidates memory.(3) Sleep duration decreases with age,

and to maintain optimal health, the American Academy of Sleep Medicine (AASM) recommends that children aged 3–5 years old obtain 10–13 hours of sleep per day, including naps.(3,4) Nevertheless, insufficient sleep remains common among preschool children worldwide and in Indonesia, and has been linked to cognitive impairments, behavioral problems, and increased risk of obesity.(5–9)

Data from the National Survey of Children's Health (NSCH) in 2016–2018 reported that 34,8% of children aged 3–5 years old had short sleep duration, increasing to 35.4% in 2019–2020.(5) Approximately 20–40% of preschoolers experience sleep disturbances.(6) In Indonesia, among 153 children with sleep problems, 16% slept less than 9 hours, and 62 children reported more than three nocturnal awakenings.(7)

Beyond behavioral and environmental factors, nutrition plays a critical role in sleep regulation. Omega-3 fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are integral components of neuronal membranes and contribute to melatonin synthesis, the key hormone regulating circadian rhythm and sleep duration.(10) DHA facilitates the conversion of serotonin into melatonin, while EPA modulates neurotransmitter release, thereby influencing sleep processes.(11) Previous studies have shown that higher intake or supplementation of omega-3 fatty acids is associated with improved sleep quality and longer sleep duration.(12) Despite growing global evidence, data on omega-3 status and its association with sleep duration among Indonesian preschoolers remain scarce. Therefore, this study aimed to analyse the association between omega-3 fatty acids levels and sleep duration among preschool children aged 3–5 years old in North Sulawesi, Indonesia.

Methods

Subject Recruitment

This study was nested within a randomized controlled trial (RCT) investigating the effect of omega-3 fatty acid supplementation on sleep quality in children aged 3–5 years old. A cross-sectional survey was conducted in kindergartens and early childhood education centres in Mapanget District, Manado City, and Kalawat District, North Minahasa Regency, between March and December 2025. The selection of study sites was determined based on similarities in sociodemographic conditions among the sites' population. Baseline data collected prior to intervention were analysed.

Sample size was calculated to meet RCT requirements with 95% confidence and 80% power. Subjects were recruited consecutively according to the inclusion and exclusion criteria, and 72 were enrolled. Inclusion criteria were children aged 3–5 years old, cared for by literate parents and willing to sign informed consent. Exclusion criteria included severe malnutrition (<-3SD), physical or intellectual disabilities, and history of asthma or allergies.

Sociodemographic Data Collection

Interviews were conducted to obtain demographic data, including child age and sex, parental age, parental education and occupation. Parental education was classified as school graduate and middle school or lower graduate.

Anthropometry Measurement

Anthropometric measurements were performed to assess nutritional status using body mass index-for-age.(13) Body weight was measured to the nearest 0.1kg using digital SECA instrument (SECA, Hamburg, Germany). Height was measured to the nearest 0,1cm using SECA stadiometer.

Sleep Quality Assessment

Sleep duration was defined as the total time spent asleep during both daytime and nighttime. Data were obtained using the Children's Sleep Habits Questionnaire-Abbreviated (CSHQ-A), which also assessed sleep disturbances across four domains: nighttime sleep, sleep behavior, nocturnal awakenings, and morning awakenings. A cut-off score of <41 indicated no sleep disturbance, while ≥ 41 indicated sleep disturbance.(14)

Gas Chromatography–Mass Spectrophotometry (GC-MS) Analysis

Laboratory assessment using GC-MS were performed to determine omega-3 fatty acid levels, including α -linolenic acid (ALA), EPA, and DHA. Analyses were conducted at Prodia Clinical Laboratory, Jakarta. Subjects fasted for 12–14 hours prior to venous blood collection. A 3 mL sample was obtained from the cubital fossa using standard aseptic techniques. Serum was separated and stored frozen until analysis. Five- hundreds μ L of serum samples were extracted with a chloroform–methanol mixture, and nonadecanoic acid (C19:0) was added as an internal standard for quantitative calibration. Free fatty acids were derivatized into fatty acid methyl esters (FAMES) using tetramethylammonium hydroxide (TMAH) in methanol. The FAME samples were injected into a polar GC column under programmed temperature conditions to separate

ALA, EPA, and DHA. Detection was performed using an Agilent 7890 GC coupled with a 5977A MSD. Identification was achieved by comparison with the FAME Standard Mix (Supelco, Bellefonte, PA), and quantification was determined from peak area ratios relative to the internal standard, with results expressed as concentrations of each fatty acid.(15,16)

Statistical Analysis

Data analysis was performed using SPSS version 26 (IBM Corporation, Armonk, NY, USA). Subject characteristics were described using univariate analysis, with categorical data presented as frequencies. The normality of numerical data was assessed using the Kolmogorov–Smirnov test. Normally distributed data were expressed as mean±standard deviation (SD), while non normally distributed data were expressed as median (minimum–maximum). Comparisons of omega 3 fatty acid levels between sleep duration groups were conducted using the Independent Samples t test for normally distributed data and the Mann–Whitney test for non normally distributed data. Receiver Operating Characteristic (ROC) curve analysis was performed to evaluate the discriminatory ability of omega 3 fatty acid levels, with the Area Under the Curve (AUC) reported. Spearman’s rank correlation was used to assess associations between omega 3 fatty acid levels and sleep duration. Analyses were conducted at a 95% confidence level, and $p < 0.05$ was considered statistically significant.

Results

Characteristics and Omega-3 Fatty Acid Profiles of Study Subjects

Median age of the study subjects was 61 months (range: 36–70 months), and 42% were male. Approximately 94.4% of subjects had normal nutritional status. Most parents were high school graduates (Table 1).

The mean total omega-3 fatty acids concentration was 225.47 ± 80.9 $\mu\text{mol/L}$, while median of ALA, EPA, and DHA were 13.5 $\mu\text{mol/L}$, 15.0 $\mu\text{mol/L}$, and 183.5 $\mu\text{mol/L}$, respectively (Table 2). Distribution of omega 3 fatty acids profile levels, as illustrated by the boxplots in Figure 1, demonstrated distinct patterns across variables. The distribution of total omega 3 fatty acids appeared relatively symmetric, with most participants within the normal reference range (100–500 $\mu\text{mol/L}$), and no prominent outliers were observed. Approximately 75% of participants

Table 1. Demographic characteristics of subjects.

Variable	Values (n=72)
Age, median (min–max)	
Subjects (month)	61 (36–70)
Paternal (year)	36.06 (24–59)
Maternal (year)	32 (22–50)
Gender, n (%)	
Male	30 (41.7)
Female	42 (58.3)
Nutritional status, n (%)	
Normal	68 (9.4)
At risk of overweight	2 (2.8)
Obese	2 (2.8)
Paternal educational status, n (%)	
High school graduate	62 (86.1)
Middle school/lower graduate	10 (13.9)
Maternal educational status, n (%)	
High school graduate	66 (91.7)
Middle school/lower graduate	6 (8.3)
Paternal employment status, n (%)	
Both parents employed	21 (29.2)
Paternal employment	51(70.8)

had ALA levels below the normal reference range (20–120 $\mu\text{mol/L}$), while several outliers were observed at higher values. EPA levels tended to be low but remained within the normal range. The presence of extreme outliers indicated that some participants had markedly higher EPA levels compared to the majority. In contrast, the distribution of DHA levels showed that most participants had values above the normal reference range (30–160 $\mu\text{mol/L}$).

Subjects’ Sleep Duration and Sleep Disturbances Scores

Based on CSHQ-A scores, sleep disturbances were highly prevalent, affecting 93.1% of subjects, with a median total disturbance score of 52. Nighttime sleep duration ranged from 7.5 to 12 hours, while daytime sleep duration was

Table 2. Total omega-3 fatty acids, ALA, EPA, and DHA concentrations of study subjects.

Variable	Values (n=72)
Total omega-3 fatty acids ($\mu\text{mol/L}$) ^a	225.47 ± 80.9
ALA ($\mu\text{mol/L}$) ^b	13.5 (3–53)
EPA ($\mu\text{mol/L}$) ^b	15.0 (3–60)
DHA ($\mu\text{mol/L}$) ^b	183.5 (31–401)

^aData presented as mean±SD; ^bData presented as median (min–max).

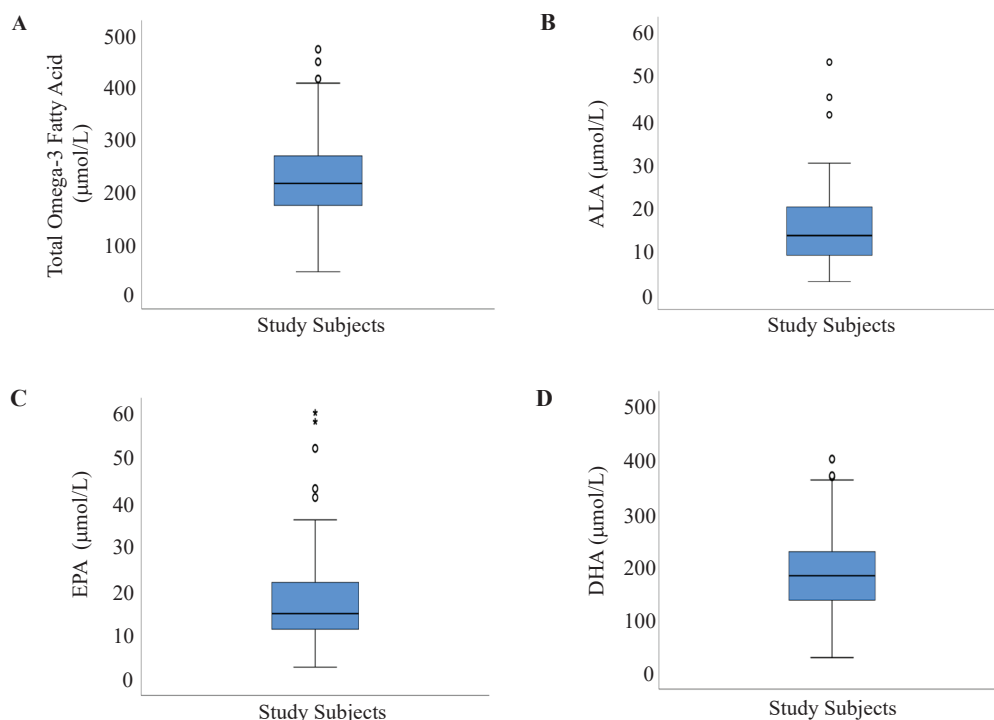


Figure 1. Distribution of omega-3 fatty acid profiles. A: total omega-3 fatty acids concentration; B: ALA concentration; C: EPA concentration; D: DHA concentration. ^oOutliers (a data points located outside the whiskers of the box plot). *Extreme outliers.

approximately 2 hours. The median total sleep duration was 11 hours (range 9–13.5 hours). Among the CSHQ-A subscales, bedtime routines had the highest mean scores, whereas waking during the night had the lowest (Table 3).

Majority of Subjects had Optimal Sleep Duration

The distribution of omega-3 fatty acid concentrations between children with optimal and non-optimal sleep duration was presented in Table 4. The majority of subjects

Table 3. Sleep duration and sleep disturbances score of the study subjects.

Variable	Values (n=72)
CSHQ-A scores	
Bedtime ^a	26.81±4.81
Sleep behavior ^b	14 (9–20)
Waking during the night ^b	3 (2–8)
Morning wake up ^b	6 (4–13)
Total sleep disturbances ^b	52 (36–63)
Daytime sleep duration (hours) ^b	2 (1–3)
Nighttime sleep duration (hours) ^b	9.5 (7.5–12)
Total sleep duration (hours) ^b	11 (9–13.5)
Sleep disturbances ^c	
Yes	67 (93.1%)
No	5 (6.9%)

^aData presented as mean±SD; ^bData presented as median (min–max); ^cData presented as n (%).

had optimal sleep duration, defined as 10–13 hours per day, while only six children had sleep duration of less than 10 hours or greater than 13 hours. No significant differences were observed for total omega-3, ALA, EPA, or DHA concentrations between the two categories ($p>0.05$).

ROC Analysis of Omega-3 Fatty Acids and Sleep Duration

ROC analysis of total omega-3 fatty acids, ALA, EPA, and DHA showed that all parameters had AUC values close to 0.5, reflecting poor discriminative ability in classifying sleep duration categories. Although ALA demonstrated perfect specificity, its sensitivity was very low. The presence of ties between groups further suggests overlapping distributions of omega-3 concentrations across sleep categories. Overall, none of the fatty acid profiles reached acceptable predictive performance (Figure 2, Table 5).

Omega-3 fatty Acids Profile was Correlated with Total Sleep Duration

Correlation analysis was performed, and significant positive correlations were observed for total omega-3 fatty acids ($r=0.298$; $p=0.011$), EPA ($r=0.233$; $p=0.049$), and DHA ($r=0.260$; $p=0.028$). In contrast, ALA showed no significant correlation with sleep duration (Table 6). These findings indicate that higher concentrations of total omega-3, EPA, and DHA were modestly associated with longer sleep duration.

Table 4. Distribution of omega-3 fatty acid concentrations according to sleep duration.

Omega-3 Fatty Acid Profiles ($\mu\text{mol/L}$)	Sleep Duration		<i>p</i> -value
	Optimal (n=66)	Not optimal (n=6)	
Total Omega-3 fatty acid, mean \pm SD	225.41 \pm 82.77	226.17 \pm 62.2	0.983 ^a
ALA, median (min–max)	13.5 (3–53)	14 (7–19)	0.846 ^b
EPA, median (min–max)	14 (3–60)	16.5 (10–58)	0.298 ^b
DHA, median (min–max)	187.5 (31–401)	176.5 (120–260)	0.959 ^b

^aTested with Independent-samples T-Test; ^bTested with Mann-Whitney U Test.

Discussion

Sleep duration and health have a bidirectional relationship, where both short and long sleep can have adverse effects. Short sleep duration may impair physical health, cognitive performance, and child behavior.(17) In this study, preschool children achieved total sleep duration within the American Academy of Sleep Medicine recommendation (10–13 hours/day).(4) However, CSHQ-A scores indicated that most children experienced sleep disturbances, with a median score of 52 exceeding the cutoff ≥ 41 . This suggests that despite adequate sleep duration, sleep quality remained poor. These findings are consistent with previous studies in Indonesia (18,19), although the prevalence of disturbances in our study was higher.

Sleep duration is one objective aspect of sleep quality, alongside sleep onset, latency, efficiency, and wake after sleep onset (WASO).(20) Sleep quality can be influenced by endogenous and exogenous factors. In addition to genetic predisposition, environmental, lifestyle, and psychosocial factors also play a role.(21) Based on a result of study in

children aged 3–5 years old, it was reported that increased screen time was associated with sleep problems. Electronic media use may heighten physiological, cognitive, and emotional arousal, reducing relaxation and delaying sleep onset.(22) Family income, maternal education, bed sharing, and gadget use within two hours before bedtime were linked to sleep problems.(23) Data from National Survey of Children's Health (NSCH) also showed that consistent bedtime routines determine adequate sleep in children. (24) Environmental conditions such as noise, lighting, and room temperature further affect sleep quality, while medical conditions including severe malnutrition, asthma, and allergic rhinitis may contribute to difficulties initiating and maintaining sleep.(25)

Sleep regulation changes rapidly during early childhood. Children aged 3–5 years old typically show monophasic sleep patterns.(25) Napping usually ceases by age 5, accompanied by reduced nighttime sleep due to delayed bedtime, while wake-up times remain unchanged. (3) Sleep disturbances are common in preschoolers, with 15–30% experiencing bedtime resistance and night awakenings.(26)

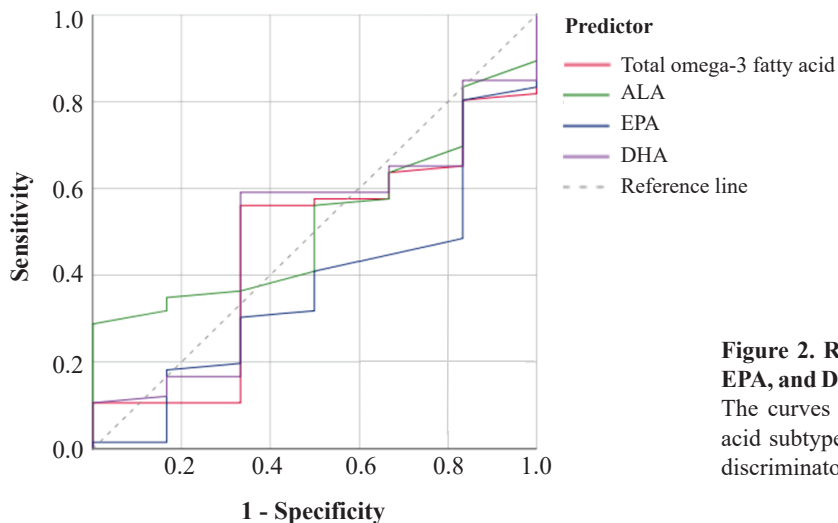


Figure 2. ROC curves of total omega-3 fatty acids, ALA, EPA, and DHA concentrations in relation to sleep duration. The curves illustrate the discriminatory ability of each fatty acid subtype. AUC values were below 0.60, indicating weak discriminatory performance.

Table 5. ROC analysis of omega-3 fatty acids for sleep duration.

Variable	AUC	95% CI	Cut-off Value (μmol/L)	Sensitivity (%)	Specificity (%)
Total omega-3 fatty acid	0.467	0.237–0.697	207.5	56.1	66.7
ALA	0.524	0.340–0.708	19.5	28.8	100
EPA	0.371	0.156–0.587	25.5	18.2	83.3
DHA	0.494	0.266–0.721	178.5	59.1	66.7

In this study, total omega-3 fatty acid concentrations were within reference ranges, but ALA was below reference values. EPA levels tended to be low but remained normal, whereas DHA exceeded the upper limit. This profile indicates an imbalance, with DHA dominating over ALA and EPA. Omega-3 fatty acids are essential nutrients obtained from diet. ALA serves as a precursor for EPA and DHA, but only a small fraction is converted.(27) After intake, intracellular ALA decreases due to dihydrolipoic acid (DHLLA) activity. ALA is largely utilized via β -oxidation, resulting in low bioavailability. In contrast, DHA has better bioavailability, as it is not a suitable substrate for β -oxidation in peroxisomes or mitochondria.(28) Conversion of ALA to EPA competes with linolenic acid conversion to arachidonic acid, since both processes involve the same enzymes.(27) Overall, DHA accumulates in the body at about twice the level of ALA. After consumption, 59% of ALA is lost, 21.2% accumulates, 0.4% is secreted, and the remainder is converted into EPA, DPA, and DHA.(29)

The ROC findings suggest that total omega-3 fatty acid, ALA, EPA, and DHA cannot be used to establish clinically meaningful cut-off points for optimal sleep duration in this sample. Although exploratory cut-off values could be generated, their predictive validity is limited due to poor discriminative ability ($AUC < 0.5$). Group comparison analysis did not show significant differences in omega-3 concentrations between children with optimal and non-optimal sleep duration. These limitations are

likely attributable to the small number of children in the non-optimal sleep group ($n=6$), which reduces statistical power and stability of ROC estimates. Therefore, the results should be interpreted cautiously and considered exploratory rather than confirmatory. To date, no cut-off values for omega-3 fatty acids, particularly EPA and DHA, have been established to determine their influence on sleep duration and quality. Nevertheless, correlation analysis revealed weak but positive associations between total omega-3, EPA, and DHA with sleep duration. These findings are not contradictory: group comparison tests evaluate mean differences between categories, whereas correlation analysis assesses linear associations across the entire dataset. The correlation results suggest a potential trend consistent with previous studies.

The Docosahexaenoic Acid Oxford Learning and Behaviour (DOLAB) study in UK children aged 7–9 years, where higher DHA status was significantly associated with better sleep. DHA supplementation was shown to increase sleep duration by 58 minutes, reduce night awakenings, and improve sleep efficiency. In this study, fatty acids were measured using the finger-stick method. Concentrations were analyzed by gas chromatography of total lipids in capillary whole blood and results were expressed as weight percentage.(30) In contrast, the Fish Intervention Studies-KIDS (FINS-KIDS) study in children aged 4-6 years found that fatty fish consumption increased EPA and DHA levels compared to meat, but no differences in sleep outcomes were observed. In this study, the fatty acid composition of total red blood cells (RBC) was determined using ultrafast gas chromatography (UFGC).(31) Additional National Health and Nutrition Examination Survey (NHANES) data indicated that shorter sleep duration was associated with lower EPA, DHA, and total omega-3 levels. In this study, serum fatty acids were analyzed with modified methods including hexane extraction and the use of isotopically labelled fatty acids as internal standards to account for recovery. Fatty acids were separated by injection on a capillary gas chromatograph column. Omega-3 fatty acids were expressed as a percentage of total fatty acids.(32)

Table 6. Correlation between omega-3 fatty acids profile and total sleep duration.

Variable	Total Sleep Duration	
	<i>r</i>	<i>p</i>
Total omega-3 fatty acid	0.298	0.011*
ALA	0.141	0.238
EPA	0.233	0.049*
DHA	0.260	0.028*

Data was analysed with Rank Spearman correlation test, *Statistically significant, $p < 0.05$.

Improved sleep quality through regular fish oil consumption has also been documented in population-based studies, with higher weekly intake associated with better sleep. In this study, omega-3 concentrations were additionally assessed through dietary intake. The number of fish servings per week was calculated by dividing the average intake of each fish by the standard portion size of 140 g. Individuals with good sleep quality reported higher weekly consumption of oily fish compared to those with poor sleep quality.(12) Furthermore, supplementation with EPA and DHA in young adults increased blood levels and extended sleep duration. In this study, RBC fatty acid composition was analyzed using gas chromatography, results were expressed as weight percentage of the total, and the n-3 index was calculated as the sum of %EPA and %DHA.(33)

Bivariate analysis showed no correlation between ALA and sleep duration, possibly because ALA is indirectly related to sleep disturbances. Some studies reported that ALA supplementation may reduce anxiety and depression symptoms.(34,35) Our findings are consistent with the DOLAB study in the UK (29), whereas NHANES data in the US showed a negative correlation between ALA and sleep duration.(36) Direct evidence of ALA's role in sleep regulation remains limited, as most research focuses on EPA and DHA.

Omega-3 fatty acids, particularly EPA and DHA, are integral components of cell membranes.(30) Animal studies reported that EPA and DHA can alter pineal gland phospholipid composition, modulating melatonin production and sleep regulation. Higher DHA levels are associated with increased melatonin concentrations, as DHA is required for serotonin-to-melatonin conversion.(37) EPA supports serotonin release by inhibiting prostaglandin synthesis, which otherwise suppresses serotonin release.(32) DHA acts as a structural component of neuronal membranes and enhances arylalkylamine N-acetyltransferase (AANAT) activity, the rate-limiting enzyme in melatonin synthesis. This mechanism facilitates serotonin conversion to melatonin, supporting circadian rhythm regulation and improving sleep efficiency. EPA exerts anti-inflammatory effects and modulates cytokine activity, reducing systemic inflammation that may disrupt sleep. EPA also contributes to mood stabilization, indirectly improving sleep quality. Together, DHA and EPA provide synergistic benefits in sleep regulation.(32,33)

To our knowledge, this study is among the first in Indonesia to assess omega-3 fatty acid levels in preschool children and their relationship with sleep duration.

Limitations include reliance on parent-reported sleep data, which may introduce recall bias, and the cross-sectional design, which restricts causal inference. Selection bias may also exist due to consecutive recruitment, limiting sample representativeness. Environmental, screen time, and psychosocial factors were not analysed. Future studies should employ objective measures such as actigraphy, adopt longitudinal or interventional designs to strengthen causal inference, and use random sampling across multiple schools to improve generalizability. Incorporating environmental, behavioral, and psychosocial factors would provide a more comprehensive understanding of the relationship between omega-3 fatty acids and sleep outcomes in preschool children.

Conclusion

This study demonstrated that total omega-3 fatty acids, EPA, and DHA levels were positively associated with sleep duration in preschool children, although correlations were weak. The findings provide preliminary evidence of the role of EPA and DHA in sleep regulation during early childhood and emphasize the importance of considering nutritional factors, particularly omega-3 intake, in efforts to improve sleep quality among young children.

Acknowledgments

The authors receive grants from the Indonesian Education Scholarship, Center for Higher Education Funding and Assessment, and the Indonesian Endowment Fund for Education.

Authors Contribution

MDA conceptualized the study, designed the methodology, collected and analysed data, interpreted findings, and drafted the manuscript. RS provided supervision, critical guidance on study design, and contributed to data interpretation. DNC assisted in refining the methodology, supported data analysis, and critically reviewed the manuscript. NM contributed to data interpretation, provided academic input, and reviewed the manuscript for intellectual content. All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Ethical Statement

Ethical approval was obtained from the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia, and Dr. Cipto Mangunkusumo National General Hospital (No: KET-42/UN2.F1/ETIK/PPM.00.02/2025).

Conflict of Interest

The authors declare no conflict of interest.

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