Annals of Phytomedicine 14(1): 993-1002, 2025

DOI: http://dx.doi.org/10.54085/ap.2025.14.1.99

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Impact of maternal dietary polyunsaturated fatty acids (PUFA) status on fatty acid profile of erythrocytes and breast milk of Punjabi women

Richika Gupta◆, Kiran Bains, Renuka Aggarwal, Sukhinder Cheema* and Aditi Sewak
Department of Food and Nutrition, Punjab Agricultural University, Ludhiana-141004, Punjab, India
**Department of Biochemistry, Memorial University of Newfoundland, Canada*

Article Info

Article history
Received 27 February 2025
Revised 13 April 2025
Accepted 14 April 2025
Published Online 30 June 2025

Keywords
LCPUFA
Docosahexaenoic acid
Arachidonic acid
Fatty acid profile
Breast milk
Lactation
Maternal nutrition

Abstract

Long-chain polyunsaturated fatty acids (LCPUFAs), particularly arachidonic acid (AA) and docosahexaenoic acid (DHA), play a critical role in fetal brain and retinal development during pregnancy and lactation. This study aimed to evaluate the dietary intake of omega-3 and omega-6 fatty acids among 50 lactating women from Ludhiana, India, and its impact on their erythrocyte fatty acid profile and DHA levels in breast milk at the first and sixth month postpartum. The findings revealed a significant decline (47.36%) in DHA levels in breast milk over the study period, despite consistent dietary intake. Conversely, omega-6 fatty acids, particularly AA and linoleic acid, remained stable in erythrocytes and breast milk. The low dietary intake of omega-3 precursors (alpha-linolenic acid, ALA) and negligible consumption of DHA-rich foods such as seafood contributed to this imbalance. Competitive inhibition between omega-3 and omega-6 pathways further exacerbated the decline in DHA levels. Maternal BMI, dietary patterns, and parity were identified as additional factors influencing LCPUFA levels. These results underscore the need for nutritional interventions and policy measures to improve omega-3 intake during pregnancy and lactation, particularly in regions with limited access to DHA-rich foods, to optimize maternal and neonatal health outcomes.

1. Introduction

In recent years, extensive research has emphasized the critical role of essential fatty acids (EFAs) in maternal and neonatal health, particularly during pregnancy and lactation as antioxidant-rich foods in diet have the potential to reduce the risk of many diseases (Aswany *et al*., 2023). Long-chain polyunsaturated fatty acids (LCPUFAs), including arachidonic acid (AA) and docosahexaenoic acid (DHA), are essential for fetal development due to their involvement in various physiological and metabolic processes (Brenna and Lapillonne, 2009). While EFAs such as linoleic acid (LA) and alpha-linolenic acid (ALA) serve as precursors to LCPUFAs, they cannot be synthesized endogenously, making dietary intake vital (Ballard and Morrow, 2013). Being a major source of antioxidants, the ALA (alpha-linolenic acid) possesses anti-inflammatory properties (Subrahmaniyan *et al*., 2024). One important class of polyunsaturated fatty acids necessary for preserving human health and well-being is omega-3 fatty acids (Saidaiah *et al*., 2024). Among these, DHA plays a pivotal role in the structural and functional integrity of the phospholipid bilayers in neural and retinal cell membranes, highlighting its significance in neurodevelopment (Innis, 2014).

During the third trimester of pregnancy, the accretion of polyunsaturated fatty acids (PUFAs) in the fetal brain accelerates, with approximately 50-70 mg of DHA accumulating daily, particularly 67 mg/day during this critical period (Innis, 2003). In addition to fetal requirements, maternal DHA needs to increase due to the expansion of red blood cell mass and placental development, with a recommended dietary intake of 300 mg/day, including 200 mg/day of DHA (Koletzko *et al*., 2008). For lactating mothers, DHA supplementation is equally crucial, as it supports neonatal cognitive and neural development through the breast. Omega-3 fatty acids, beyond their role in neural development, also offer cardioprotective, lipid-lowering, and anti-inflammatory effects, and extend gestational duration, reducing the risk of preterm birth (Cicero *et al*., 2016) due to their anti-inflammatory properties which modulates the synthesis of prostaglandins and supports the placental functions.

India faces a significant public health challenge, with approximately 27 million births annually, of which 3.6 million are preterm, contributing to 300,000 neonatal deaths each year (Blencowe *et al*., 2013). The scarcity of omega-3 PUFA-rich foods, reliance on plant-based diets, and limited nutrition education contribute to these statistics. Further, a massive number of chemical pesticides are employed for crop protection (Elanselvi *et al*., 2024). In Punjab, while the consumption of LA-rich foods is widespread due to dietary patterns, the intake of ALA and DHA remains low, largely attributed to minimal seafood consumption. This deficiency is further exacerbated by structural, socio-cultural, and physiological barriers that limit access to balanced diets during pregnancy and lactation (Kapil *et al*., 2017).

The first 1,000 days of life, encompassing conception to a child's second birthday, represent a critical window for brain development and long-term health outcomes. DHA plays a crucial role in this period, as the neural and cognitive development of the fetus and infant is highly sensitive to maternal nutrition. This underscores the

Corresponding author: Ms. Richika Gupta
Department of Food and Nutrition, Punjab Agricultural University, Ludhiana-141004, Punjab, India
E-mail: richugarg39@gmail.com
Tel.: +91-8288885880

importance of improving maternal dietary intake of n-3 PUFAs before and during pregnancy (Black *et al*., 2013). Further, it has led to an increased interest in exploring more sustainable, alternative ways to improve human well-being (Santhosha *et al*., 2023). Therefore, this research aimed to evaluate the impact of maternal diets in Ludhiana, Punjab; during the last trimester of pregnancy and lactation on the fatty acid composition of erythrocyte and DHA levels in breast milk at baseline (after delivery) first and six months postpartum.

2. Materials and Methods

2.1 Study design and subjects

A total of 50 lactating women from the region of Ludhiana were selected from the local Anganwadi and Civil Hospital, Ludhiana. The selected subjects belonged to low and lower-middle-income groups. The inclusion criteria were age between 18-35 years, willingness to continue till the study I completed, providing the required information about herself, family, child, dietary assessment, anthropometry of herself and child, biological samples (blood and milk), filling of the questionnaire, willing to agree on the instruction of studies and exclusion criteria was women with the history of any metabolic disorder such as diabetes, hypertension, cardiovascular disease or diseases like cancer, thyroid, liver and bleeding problem, severe anaemia or thrombosis or diseases that had a direct link of causing health concerns to both mother and offspring, women consuming n-3 supplementation, high-risk pregnant mothers.

After fulfilling the inclusion criteria, the recruited women were informed about the research, its objective, the purpose of taking their breast milk (as there is a stigma in society on giving their milk), and general information about the study. The protocol was approved by the Ethics Committee of the Clinical hospital, Punjab Agricultural University, Ludhiana, and permission was taken from the Civil Surgeon of Ludhiana, Dr. Jasbir Singh Aulakh to work in Civil Hospital. Each enrolled participant was thoroughly informed about the study being conducted and consent forms were signed by them.

2.2 Clinical and anthropometric assessment

A comprehensive clinical and anthropometric assessment was conducted following the enrolment of participants in the study. A trained nurse evaluated the health status of each lactating woman, specifically determining whether lactation had commenced, identifying any difficulties in breastfeeding, and performing standard clinical assessments for lactating mothers. Anthropometric measurements, including height and weight, were taken following standardized procedures. Waist and hip circumferences were measured by the methods outlined by the World Health Organization (WHO, 2008). Using the collected data, key metrics such as body mass index (BMI, kg/m²), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated.

Similarly, infants underwent anthropometric assessments, with body weight, length, and head circumference measured using standard protocols. The study adhered to guidelines and procedures prescribed by the WHO (2008) and the Indian Council of Medical Research (ICMR, 2020) to ensure accuracy and uniformity in data collection.

2.3 Dietary intake

After taking the sociodemographic information of the enrolled subjects, they were asked to duly fill the food frequency questionnaires (FFQ) (indicating foods rich in n-3 fatty acids) that were consumed by them during their pregnancy, 1st and 6th month postpartum. To be precise in the amounts/ quantity, a photographic atlas as given in Dietcal Software was shown to them. Two days semi-quantitative dietary recall method was used to estimate the food consumed at 1st and 6 months postpartum. The information so obtained was recorded in Dietcal software to obtain their daily intake levels of macro and micronutrients and were then compared with the RDA (ICMR, 2020).

2.4 Collection of milk and blood samples for fatty acid analysis

Blood and milk samples were taken from the enrolled mothers at 1st and 6 months postpartum. Dried blood spots (DBS) and Dried milk spots (DMS) cards having polymer or cellulose paper were used for the collection of samples. For the collection of blood samples, a finger was pricked by a sterilized non-reusable pin, then a drop of blood was collected on the card, followed by air drying and transferring it into the sealed bags for easy transport. Similarly, 5-6 drops of milk were collected in a plastic cup which was then spotted on the card with a dropper. The obtained samples were then sent to Lipomic Lab, Delhi for further analysis of the complete fatty acid profile of blood and DHA estimation of milk samples.

2.5 Statistical analysis

Descriptive analysis was performed to obtain the baseline characteristics of the participants. Frequency distribution and cross-tabulation were performed to prepare the tables. The data was expressed as means and standard deviations (continuous variables) or as numbers and percentages (categorical variables). All statistical analyses were performed using SAS 9.3 software (SAS Institute Inc.), and $p<0{\cdot}0001$ was considered significant.

3. Results

3.1 Sociodemographic background and anthropometric characteristics of the respondents

The baseline characteristics, anthropometric measurements, and nutritional status of 50 lactating mothers were evaluated and compared with existing research. The mean age of the mothers in this study (23.5 ± 4 years) aligns with findings from similar studies in low and middle-income countries (LMICs) reporting an average maternal age of 20-25 years (Black *et al*., 2013). Educational attainment showed that nearly half (48%) had only primary education, with only 8per cent being college graduates, highlighting limited educational opportunities, which is a known barrier to maternal and child health (Victora *et al*., 2016). The majority of mothers (62%) were homemakers, while 38 per cent were working, which reflects shifting socioeconomic dynamics in some LMICs. Dietary habits revealed 54 per cent of mothers were vegetarians, consistent with regional dietary preferences in South Asia, but studies suggest that vegetarian diets during lactation require careful monitoring to ensure adequate protein, iron, and vitamin B-12 intake (Rogerson *et al*., 2018).

Postpartum anthropometric measurements revealed significant weight and BMI reductions. Mean weight dropped from 56.82 ± 5.94 kg after delivery to 49.48 ± 4.42 kg at six months postpartum ($p<0.0001$), with a corresponding decrease in BMI from 23.38 ± 2.45 kg/m² to 20.36 ± 1.81 kg/m². These trends are consistent with Butte *et al*. (2004), who reported that lactation-associated energy demands often result in postpartum weight loss, particularly in breastfeeding mothers. However, the increase in underweight mothers from 4 per cent at one month postpartum to 10 per cent at six months indicates potential undernutrition, reflecting findings from Black *et al*. (2013) that inadequate maternal nutrition during lactation can exacerbate energy and nutrient deficiencies. Additionally, the decline in overweight mothers from 18 per cent after delivery to 14 per cent at six months may suggest improved metabolic adjustments postpartum, which has also been noted in lactating women (Victora *et al*., 2016).

The study's dietary data showed 16 per cent of mothers consumed fish or seafood, a key source of omega-3 fatty acids critical for lactation, but lower than global averages, raising concerns about DHA intake, which is essential for infant neurodevelopment (Koletzko *et al*., 2008).

Table 1: Baseline (after delivery) characteristics of mothers

	n-50
Age group (years), Mean ± S.D	23.5 ± 4
Education	
College graduate/above, n (%)	04 (8%)
High school/secondary, n (%)	15 (30%)
Primary education	24 (48%)
Uneducated, n (%)	07 (14%)
Homemaker, n (%)	19 (38%)
Working, n (%)	31 (62%)
Household income	
(≥10,000 INR), n (%)	04 (18%)
(≥15,000), n (%)	39 (78%)
(≥20,000), n (%)	07 (14%)
No. of children	
1st child, n (%)	27 (54%)
2nd child, n (%)	18 (36%)
3rd child, n (%)	05 (10%)
No. of stillbirths/miscarriages in previous pregnancy, n (%)	07 (14%)
Dietary habits	
Vegetarian, n (%)	27 (54%)
Ovo-vegetarian, n (%)	13 (26%)
Non-vegetarian, n (%)	10 (20%)
Consuming fish/seafood, n (%)	08 (16%)

Values are Mean ± S.D, or as a percentage (%)

Table 2: Anthropometric measurements of mothers

	After delivery (within a week)	1 month postpartum	6 months postpartum	*p*-value
Height (cm), mean ± S.D	156.1 ± 5.6	156.1 ± 5.6	156.1 ± 5.6	-
Weight (kg), mean ± S.D	56.82 ± 5.94 ^{a}	50.64 ± 2.87 ^{b}	49.48 ± 4.42 ^{b}	<0.0001
BMI (kg/m^2), mean ± S.D	23.38 ± 2.45 ^{a}	20.84 ± 1.18 ^{b}	20.36 ± 1.81 ^{b}	<0.0001

Values are Mean ± S.D; values with different alphabets in superscripts differ significantly ($p \leq 0.0001$).

Table 3: Nutritional status of mothers

	After delivery (within a week)	1 month postpartum	6 months postpartum
Underweight (%), n	-	4, 2	10, 5
Normal weight (%), n	78, 39	80, 40	76, 38
Overweight (%), n	18, 9	16, 8	14, 7
Obese (%)	4, 2	-	-

Values are as percentage.

3.2 Baseline and anthropometric characteristics of infant

The study assessed baseline characteristics, feeding practices, and anthropometric measurements of 50 infants. The median gestational age at delivery was 39.0 weeks (IQR: 38.0-40.0), aligning with the World Health Organization (WHO) definition of term pregnancies, which ranges from 37 to 42 weeks (WHO, 2006). Most infants (84%) were delivered in hospitals, while 16 per cent were delivered at home by Asha workers, consistent with studies highlighting increasing institutional delivery rates in developing regions due to government initiatives such as Janani Suraksha Yojana in India (Lim *et al*., 2010). However, the proportion of home deliveries reflects ongoing challenges in achieving universal access to institutional care (Table 4). Early breastfeeding initiation within the first hour was observed in 34 per cent of infants, lower than the global average of 48 per cent reported by UNICEF (2023), highlighting the need for improved awareness and support for breastfeeding practices. Exclusive breastfeeding until six months was achieved in only 30 per cent, below the WHO global target of 50 per cent and consistent with studies identifying maternal workload, sociocultural practices, and insufficient breastfeeding support as barriers (Black *et al*., 2013; Victora *et al*., 2016) (Table 5). Complementary feeding was initiated at a mean age of 5.5 ± 0.77 months, aligning with WHO recommendations for introducing solids at six months (WHO, 2003).

Anthropometric measurements demonstrated significant growth in the cohort. Mean length increased from 48.16 ± 2.6 cm after delivery to 67.12 ± 3.35 cm at six months, while mean weight rose from 2.72 ± 0.43 kg to 5.7 ± 0.66 kg, and mean head circumference grew from 34.01 ± 0.77 cm to 37.32 ± 0.43 cm during the same period (Table 6). These values align with WHO child growth standards (WHO, 2006), indicating overall healthy growth. However, the relatively low rates of early and exclusive breastfeeding could impact long-term growth and immunity, consistent with findings linking breastfeeding to better nutritional outcomes and reduced morbidity in infancy (Horta *et al*., 2015). This study highlights positive trends in institutional deliveries and growth patterns but underscores the need for targeted interventions in breastfeeding practices and maternal education to optimize infant health outcomes.

Table 4: Baseline (after delivery) characteristics of infants

Gestational age at the time of delivery (weeks), mean ± S.D	39.0 ± 1
Place of delivery	
Hospital, n (%)	42 (84%)
At home by Asha workers, n (%)	08 (16%)
Delivery method	
Spontaneous labour, n (%)	27 (54%)
Cesarean, n (%)	23 (46%)

Values are mean ± S.D; or as a percentage,

Table: 5 Feeding practices

	N=50
Started breastfeeding in 1h, n (%)	17 (34%)
Age at which complementary feeding initiated (months), mean ± S.D	5.5 ± 0.77
Exclusive breastfeeding until 6 months, n (%)	15 (30%)

Values are Mean ± S.D; or as a percentage.

Table 6: Anthropometric measurements of infants

	After delivery (within a week)	1 month postpartum	6 months postpartum
Length (cm)	$48.16^{a} \pm 2.6$	$54.40^{b} \pm 2.73$	$67.12^{c} \pm 3.35$
Weight (kg)	$02.72^{a} \pm 0.43$	$03.15^{b} \pm 0.43$	$05.70^{c} \pm 0.66$
Head circumference	$34.01^{a} \pm 0.77$	$35.61^{b} \pm 0.44$	$37.32^{c} \pm 0.43$

Values are Mean ± S.D; values with different alphabets in superscripts differ significantly ($p \leq 0.0001$).

3.3 Nutrient consumption trends of respondents

The consumption frequency scores (CFS) of n-3 PUFA-rich foods among 50 pregnant and lactating mothers revealed notable trends across pregnancy, the first month postpartum, and the sixth month postpartum. Desi ghee consumption significantly increased from 7.35 ± 13.22 during pregnancy to 27.22 ± 17.89 at six months postpartum ($p<0.0001$), reflecting cultural beliefs in South Asia that ghee supports postpartum recovery and lactation (Kapil *et al*., 2017). Mustard oil, a primary regional cooking oil and a source of alpha-linolenic acid (ALA) showed high CFS during pregnancy (92.88 ± 1.23) and the first month postpartum (98.88 ± 1.18) but dropped significantly by the sixth month (70.66 ± 3.35, $p<0.0001$). This aligns with studies indicating that mustard oil consumption declines as families transition to a diverse postpartum diet (Rogerson *et al*., 2018). Conversely, the intake of hydrogenated oil, often linked to affordability, peaked at 75.55 ± 29.82 by the sixth month postpartum, despite its trans-fat content ($p<0.0001$). This trend is consistent with findings from Lim *et al*. (2010), highlighting socioeconomic influences on dietary choices in LMICs.

Nuts and oilseeds showed consistent but low consumption levels. Walnuts and almonds, both rich sources of ALA, showed no significant changes in CFS throughout (10.24 ± 5.60 and 12.20 ± 8.46 during pregnancy, respectively). However, flaxseed, a potent source of n-3 PUFAs, was consumed only during the first month postpartum (1.67 ± 3.55, p=0.0009). This limited consumption mirrors findings by Black *et al*. (2013), which emphasize the low awareness and utilization of high-nutrient foods like flaxseed in LMICs. Similarly, the negligible intake of peanuts (0.26 ± 0.56 during pregnancy, p=0.0085) and sesame seeds underscores the lack of dietary diversity, as observed by Rogerson *et al*. (2018).

The consumption of green leafy vegetables revealed inconsistent patterns. Mustard leaves (0.61 ± 0.72) and spinach (2.19 ± 0.55) were consumed moderately during pregnancy and the first month postpartum but declined sharply by the sixth month, despite their high ALA and iron content. Amaranth leaves showed a brief peak postpartum (4.53 ± 0.21, p=0.0067), possibly reflecting cultural postpartum dietary practices. This decline in green leafy vegetable intake postpartum aligns with research by Victora *et al*. (2016), which highlighted a reduction in maternal micronutrient diversity due to caregiving priorities and limited access to nutrient-rich foods.

Table 7: Consumption frequency scores (CFS) of PUFA-rich foods by pregnant and lactating mothers

Food	During pregnancy(n=50)	1st month postpartum (n=50)	6th month postpartum(n=50)	p-value
	Fats and oils			
Desi ghee	07.35 ± 13.22[a]	04.65 ± 10.99[b]	27.22 ± 17.89[c]	<0.0001
Mustard oil	92.88 ± 1.23[b]	98.88 ± 1.18[b]	70.66 ± 3.35[a]	<0.0001
Sunflower oil	03.06 ± 7.67	0	01.64 ± 2.55	0.4856
Hydrogenated oil	62.23 ± 32.43[b]	45.32 ± 26.99[a]	75.55 ± 29.82[b]	<0.0001
	Nuts and oilseeds			
Walnuts	10.24 ± 5.60	13.38 ± 2.56	12.45 ± 4.44	0.5909
Almonds	12.20 ± 8.46	15.55 ± 4.43	10.24 ± 2.98	0.6338
Sesame seeds	04.64 ± 5.45	06.67 ± 6.38	0	0.5560
Flaxseeds	0	01.67 ± 3.55	0	0.0009
Peanuts	00.26 ± 0.56	0	0	0.0085
	Green leafy vegetables			
Mustard leaves	0.61 ± 0.72	0.32 ± 0.55	0	0.0798
Spinach	2.19 ± 0.55	5.35 ± 1.13	0	0.0998
Fenugreek leaves	1.64 ± 3.54	0.52 ± 0.03	0	0.6780
Amaranth leaves	0.61 ± 0.72	4.53 ± 0.21	0	0.0067

*Consumption frequency scores: consumption days per week x consumption weeks/365 x 100; Values are Mean ± S.D; values with different alphabets in superscripts differ significantly (p≤0.0001).

The significant changes in nutrient intake between the 1st and 6th month postpartum highlight notable shifts in dietary patterns. Total fat intake increased from 28.21 ± 13.10 g to 39.61 ± 7.27 g (p=0.0413), reflecting a rise in fat consumption during lactation, which is consistent with other studies showing an increase in fat intake among lactating women to support milk production (Koletzko *et al*., 2009). Additionally, significant increases in saturated fatty acids (SFA) were observed, with values rising from 12.74 ± 4.65 g to 21.94 ± 5.96 g (p<0.0001), indicating a shift toward higher consumption of saturated fats. This trend may be due to increased consumption of fatty foods during lactation, which may not always align with recommended dietary guidelines that suggest moderation in saturated fat intake (Das, 2006). Polyunsaturated fatty acids (PUFA), particularly omega-3 and omega-6 fatty acids, a tough increase from 2.56 ± 0.26 (first month postpartum) to 3.10 ± 0.87 (6 months postpartum) but significant change was observed which indicates that with an increase in fat consumption only SFA intake has been increased. This low consumption of PUFA could be related to inadequate intake of omega-3-rich foods, such as fish, flaxseeds, or chia seeds, which are critical for maternal health and infant brain development (Brenna and Lapillonne, 2009).

3.4 Fatty acid profile of blood and breastmilk samples of selected lactating mothers

The significant changes observed in the red blood cell fatty acid profile of lactating mothers indicate several key metabolic shifts. Myristic acid (C14:0) and palmitic acid (C16:0) both showed significant increases, from 0.566 ± 0.23 to 1.53 ± 0.17 (p<0.0001) and 9.99 ± 1.03 to 12.64 ± 2.51 (p<0.0001), respectively, suggesting a rise in saturated fats during lactation (Brenna and Lapillonne, 2009). Additionally, Oleic acid (C18:1) significantly decreased from 22.42 ± 2.57 to 19.78 ± 2.09 (p<0.0001), indicating shifts in the composition of monounsaturated fatty acids (Das, 2006). However, eicosapentaenoic acid (EPA) decreased dramatically from 1.584 ± 0.06 to 0.44 ± 0.05 (p<0.0001), and docosahexaenoic acid (DHA) exhibited a sharp decline from 4.94 ± 0.68 to 1.17 ± 0.61 (p<0.0001), reflecting a significant reduction in these crucial omega-3 fatty acids, which are essential for neonatal brain development (Brenna and

Lapillonne, 2009; Koletzko *et al*., 2009). Daily nutrient intake (DNI) levels showed that PUFA levels have increased from in sixth month to the first but in particular, n-6 consumption has been increased which was seen in blood parameters and n-3 has been decreased due to its lesser consumption. The AA/EPA ratio, an inflammatory index, significantly increased from 5.84 ± 1.91 to 21.43 ± 24.2 (p<0.0001), suggesting a shift towards a more pro-inflammatory profile due to the imbalance between omega-6 and omega-3 fatty acids (Das, 2006). This shift highlights the need for dietary interventions to improve omega-3 intake and restore the omega-3/omega-6 balance to promote better maternal and neonatal health outcomes.

The milk DHA levels of selected lactating mothers are shown in Table 10 indicating a significant decline from 0.47 ± 0.16 to 0.25 ± 0.1 (p<0.0001) due to decreased consumption of n-3 fatty acids throughout the study which was also reflected in their erythrocyte profile of fatty acid.

Table 8: Daily intake of nutrients by lactating women (24 h DR)

Nutrients	1st month postpartum	6th month postpartum	*p*-value	SDI
Energy (kcal)				
Range	782.88 - 1706.32	1200.00 - 1750.00		2500*
Mean ± S.D	978.82 ± 432.76	1327.00 ± 630.06	0.6637	
Protein (g)				
Range	029.91- 56.48	0033.00 - 070.00		59**
Mean ± S.D	046.61±8.74	0052.35 ± 14.24	0.7456	
Carbohydrates (g)				
Range	114.55 - 187.69	0110.00 - 191.00		200
Mean ± S.D	137.15 ± 80.02	0176.66 ± 117.25	0.7954	
Total Fat (g)				
Range	15.56 - 34.43	20.00 - 45.00		30 g (visible)
Mean ± S.D	28.21 ± 13.10	39.61 ± 7.27	0.0413	d≤30% of TE
Total dietary fibre (g)				
Range	15.95 - 25.96	12.00 - 30.00		40
Mean ± S.D	18.32 ± 7.00	27.35 ± 6.25	0.0013	
Thiamine (mg)				
Range	00.71 - 1.64	0.71 - 2.00		0.95
Mean ± S.D	00.91 ± 0.26	0.99 ± 0.33	0.8556	
Riboflavin (mg)				
Range	00.48 - 1.32	0.50 - 1.70		1.6 (+0.5)
Mean ± S.D	01.00 ± 0.24	1.15 ± 0.29	0.9954	
Niacin (mg)				
Range	04.10 - 13.25	4.09 - 13.25		16 (+4)
Mean ± S.D	05.21 ± 4.02	5.70 ± 2.98	0.9987	
Pyridoxine (mg)				
Range	00.62 - 1.77	0.62 - 1.77		2.5 (+0.5)
Mean ± S.D	01.06 ± 0.37	1.02 ± 0.34	0.9985	
Folate (mcg)				
Range	146.70 - 466.03	146.70 - 500.00		300 (+100)
Mean ± S.D	145.69 ± 134.04	156.00 ± 141.11	0.9980	
Magnesium (mg)				
Range	250.00 - 350.00	223.17 - 388.58		310
Mean ± S.D	184.07 ± 35.34	198.96 ± 49.53	0.9990	

Calcium (mg)				
Range	300.53 - 936.61	300.53 - 940.00		1600 (+600)
Mean ± S.D	452.18 ± 168.67	542.56 ± 303.04	0.8701	
Iron (mg)				
Range	8.57 - 10.00	7.14 - 12.92		21
Mean ± S.D	9.15 ± 0.53	9.37 ± 1.60	0.9954	
SFA (g)				
Range	8.72 - 41.20	25.59 - 72.23		≤8.7; ≤11.8***
Mean ± S.D	12.74 ± 4.65	21.94 ± 5.96	<0.0001	
MUFA (g)				
Range	05.37 - 33.43	15.77 - 43.93		10.87; 14.75****
Mean ± S.D	10.00 ± 8.56	20.00 ± 7.34	0.0876	
PUFA (g)				
Range	9.85-24.66	1.52 - 7.43		8.7; 11.8*****
Mean ± S.D	2.56 ± 0.26	3.10 ± 0.87	0.456	(8% of TE)

Values are Mean ± SD, Significant difference at $p \leq 0.0001$; *1900 kcal + 600 kcal for lactating mothers; **0.83*mean weight + 19 g; ***≤8% of TE; ****10% of TE; ***** 6- 10% of TE; TE= total energy.

Table 9: Red blood cell fatty acid profile of lactating mothers

Fatty acids		1st month postpartum) (n = 30	6th month postpartum (n = 30)	*p*-value
Saturated fatty acids (SFA)				
Myristic acid (MA)	C14:0	0.566 ± 0.23	01.53 ± 0.17	<0.0001
Palmitic acid (PA)	C16:0	30.00 ± 1.91	31.13 ± 2.07	<0.0001
Stearic acid (SA)	C18:0	09.99 ± 1.03	12.64 ± 2.51	0.5002
Monounsaturated fatty acids (MUFA)				
Palmitoleic acid (PLA)	C16:1	01.30 ± 0.33	02.07 ± 0.93	0.0004
Oleic acid (OA)	C18:1	22.42 ± 2.57	19.78 ± 2.09	<0.0001
Polyunsaturated fatty acids (PUFA) - Omega 6				
Linoleic acid (LA)	C18:2	20.74 ± 2.15	32.14 ± 1.96	0.0450
Gamma-linolenic acid (GLA)	C18:3	00.59 ± 1.21	00.71 ± 0.35	0.9978
Dihomo-gamma-linolenic acid (DGLA)	C20:3	01.72 ± 0.765	01.75 ± 0.56	1.00
Arachidonic acid (AA)	C20:4	09.25 ± 1.71	11.43 ± 2.91	0.0079
Total n-6		32.41 ± 2.90	44.14 ±3.55	0.0802
Polyunsaturated fatty acids (PUFA) - Omega 3				
Alpha-linolenic acid (ALA)	C18:3	00.69 ± 0.13	00.33 ± 0.10	0.0452
Eicosapentaenoic acid (EPA)	C20:5	1.584 ± 0.06	00.44 ± 0.05	<0.0001
Docosapentaenoic acid (DPA)	C22:5	01.16 ± 0.24	00.65 ± 0.02	0.0015
Docosahexaenoic acid (DHA)	C22::6	04.94 ± 0.68	01.17 ± 0.61	<0.0001
Total n-3		06.69 ± 0.92	04.33 ± 1.03	0.6546
Dietary index (n3/n6)		00.21 ± 0.09	00.13 ± 0.03	0.8036
Inflammatory index (AA/EPA)		05.84 ±1.91	21.43 ± 24.2	<0.0001

Values are Mean ± SD.
Significant difference at $p \leq 0.0001$

Table 10: Milk DHA profile of selected lactating mothers

	1st month postpartum (n=30)	6th month postpartum(n=30)	*p*-value	per cent decrease
milk DHA	0.47 ± 0.16	0.22 ± 0.16	<0.0001	47.36

Values are Mean ± S.D, or as percentage; Significant difference at $p \leq 0.0001$.

4. Discussion

This study provides significant insights into the role of maternal diet in influencing the fatty acid composition of erythrocytes and breast milk, focusing on omega-3 and omega-6 long-chain polyunsaturated fatty acids (LCPUFAs). DHA, a critical omega-3 LCPUFA, plays a pivotal role in the neurodevelopment of the fetus and infant, as well as in maintaining maternal health. Despite its importance, this study revealed a marked decline (47.36%) in DHA levels in breast milk from the first to six months postpartum. This reduction, coupled with stable levels of omega-6 fatty acids such as arachidonic acid (AA) and linoleic acid (LA), underscores the urgent need to address dietary imbalances during pregnancy and lactation.

4.1 Omega-3 fatty acid deficiency and DHA decline

The observed decline in DHA levels is primarily attributed to inadequate dietary intake of omega-3 precursors, such as alpha-linolenic acid (ALA), and negligible consumption of DHA-rich foods like seafood, walnuts, and flaxseeds. This finding aligns with previous research indicating that vegetarian and plant-based diets, which dominate in Ludhiana, Punjab, are inherently low in DHA and ALA (Brenna and Lapillonne, 2009). ALA, the primary plant-based omega-3 fatty acid, is poorly converted into DHA in humans due to low enzymatic efficiency, with conversion rates reported as low as 0.5-5 per cent (Innis, 2014). Additionally, the high intake of omega-6 fatty acids, predominantly from mustard oil and hydrogenated fats, exacerbates the issue. Omega-6 fatty acids compete with omega-3 fatty acids for the same enzymatic pathways (desaturases and elongases), limiting the synthesis of DHA (Das, 2006).

Maternal dietary patterns in this study further revealed significant gaps. While mustard oil was the predominant fat source, omega-3-rich foods were rarely consumed, with only 16 per cent of non-vegetarian participants reporting fish intake. This is consistent with findings from Kim *et al*. (2017), who demonstrated that maternal DHA levels in breast milk strongly correlate with dietary fish and seafood intake. The absence of omega-3 supplementation among study participants further aggravated the dietary deficit. Studies have shown that supplementation with DHA or omega-3-rich oils, such as fish oil or algal oil, can significantly enhance maternal DHA levels and their transfer to breast milk (Sparkes, 2020).

4.2 Omega-6 fatty acid stability and imbalance

Unlike DHA, the levels of omega-6 fatty acids, including AA and LA, remained stable throughout the study period. This stability reflects the high consumption of omega-6-rich oils, such as mustard oil, a dietary staple in Punjab. While AA is essential for fetal growth and development, excessive omega-6 intake can lead to an imbalance in the omega-6/omega-3 ratio, promoting pro-inflammatory states (Hoge *et al*., 2018). The inflammatory index (AA/EPA ratio) in this study increased significantly over six months postpartum, consistent with findings from previous research that excessive omega-6 intake relative to omega-3 can predispose individuals to inflammatory and metabolic disorders (Das, 2006).

This imbalance is particularly concerning given the first 1,000 days of life from conception to two years of age represent a critical window for brain development (Cusick and Georgieff, 2016). DHA is preferentially incorporated into the brain and retina during this period, with an estimated 67 mg of DHA accumulating daily during the third trimester of pregnancy (Innis, 2003). A deficiency in maternal DHA intake can impair this accretion, potentially affecting long-term cognitive and visual outcomes in infants (Bazinet and Layé, 2014).

4.3 Impact of maternal BMI on fatty acid profiles

Maternal body mass index (BMI) emerged as another significant factor influencing fatty acid metabolism and breast milk composition. At baseline, 18 per cent of mothers were overweight, and 4 per cent were obese. Overweight and obesity are associated with altered lipid metabolism, including reduced activity of desaturase enzymes critical for converting ALA into DHA (Valenzuela and Videla, 2011). Moreover, overweight individuals often exhibit a pro-inflammatory state, further increasing the demand for omega-3 fatty acids to counteract inflammation (Hoge *et al*., 2018).

The observed reduction in overweight and obese mothers to 14 per cent by six months postpartum suggests a potential improvement in overall health status; however, this reduction did not translate into improved DHA levels. Similar findings have been reported in Chilean studies, where maternal BMI was inversely correlated with DHA levels in breast milk, despite dietary interventions.

4.4 Influence of parity and lactation on DHA reserves

Parity and lactation demand significantly affect maternal DHA reserves. In this study, 46 per cent of participants had at least two children, which may have contributed to the depletion of DHA stores over successive pregnancies and lactation periods. Previous research has demonstrated that DHA mobilization from maternal stores decreases with each subsequent pregnancy, especially in the absence of adequate dietary replenishment. This cumulative depletion can result in lower DHA levels in breast milk and reduced transfer to the neonate, as observed in this study.

4.5 Dietary interventions and policy recommendations

To address the nutritional gaps identified in this study, targeted dietary interventions are necessary. Increasing the intake of omega-3-rich foods, such as chia seeds, walnuts, flaxseeds, and fish, should be a priority. For populations with limited access to natural sources of DHA, omega-3 supplementation offers a viable alternative. Studies have shown that supplementation with as little as 0.7 g/day of DHA and EPA can significantly enhance maternal DHA status and its transfer to breast milk (Sparkes, 2020).

Replacing omega-6-rich oils with ALA-rich alternatives, such as flaxseed oil, could also help restore the omega-3/omega-6 balance. Additionally, maternal nutrition education programs should emphasize the importance of omega-3 intake during pregnancy and lactation. Public health policies could also promote the fortification of commonly consumed foods, such as cooking oils and milk, with DHA to address widespread deficiencies.

4.6 Limitations and future research

While this study provides valuable insights, it is not without limitations. The sample size was relatively small and limited to a specific geographic region, potentially reducing the generalizability of the findings. Future research should include larger, more diverse populations and investigate the role of genetic polymorphisms in desaturase enzyme activity, which may influence individual variability in DHA synthesis (Hoge *et al*., 2018). Additionally, longitudinal studies tracking maternal and neonatal outcomes over the first 1,000 days of life would provide a more comprehensive understanding of the long-term implications of maternal fatty acid status.

5. Conclusion

This study underscores the critical role of maternal diet in shaping the fatty acid profile of breast milk and its implications for neonatal health. The significant decline in DHA levels, driven by low omega-3 intake and high omega-6 consumption, highlights the need for targeted interventions to address these nutritional imbalances. Improving maternal DHA intake through dietary modifications, supplementation, and public health policies can optimize neonatal outcomes during the crucial early stages of development. Collaborative efforts among policymakers, healthcare providers, and researchers are essential to promote maternal and child health and reduce the burden of nutritional deficiencies.

Conflict of interest

The authors declare no conflict of interest relevant to this article.

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Citation **Richika Gupta, Kiran Bains, Renuka Aggarwal, Sukhinder Cheema and Aditi Sewak (2025). Impact of maternal dietary polyunsaturated fatty acids (PUFA) status on fatty acid profile of erythrocytes and breast milk of Punjabi women. Ann. Phytomed., 14(1):993-1002. http://dx.doi.org/10.54085/ap.2025.14.1.99.**