Erythrocyte Fatty Acid composition of Nepal breast fed infants.

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Abstract

Purpose: Essential fatty acids play a critical role in the growth and development of infants, but little is known about the fatty acid status of populations in low-income countries. The objective was to describe the fatty acid composition of red blood cells (RBC) in breastfeed Nepali infants and a subsample of their mothers and to identify the main sources of fatty acids in the mother's diet, as well as the fatty acid composition of breastmilk. Methods: RBC fatty acid composition was analyzed in a random sample of 303 infants and 72 mother, along with 68 breastmilk samples. Fatty acid profiles of the most important dietary fat sources were analyzed. Information on mother's diet and intake of fat was collected by three 24 h dietary recalls. Results: In infant RBC's, docosahexaenoic acid (DHA) was the main n-3 fatty acid and arachidonic acid (AA) was the major n-6 fatty acid. Total n-6 PUFA was three times higher than total n-3 PUFA. Height for age (HAZ) was positively associated with DHA status and AA status in multivariable models. The concentration of all fatty acids were higher in children, compared to mothers, except Total n-6 PUFA and Linoleic acid (LA) where no differences were found The mother's energy intake from fat was 13% and cooking oil (sesame, mustard, soybean or sunflower oil) contributed 52% of the fat intake. Conclusions: RBC DHA levels in both infants and mother was unexpected high taking into account few dietary DHA- sources and the low DHA concentrations in breastmilk.

Keywords: Polyunsaturated fatty acids, plasma phospholipids, DHA, AA, breast fed children, breastmilk

Introduction

Omega-3 and omega-6 fatty acids play an important role in growth and development of infants and young children [1-3]. Ensuring adequate intakes of fat, essential fatty acids (EFAs) and especially the EFA derivatives docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6) through these life stages is crucial. In low-income countries intakes of fat and especially omega-3 fatty acids is inadequate among many pregnant and lactating women [4]. Populations consuming low amounts or no animal foods will depend largely or completely on the synthesis of eicosapentaenoic acid (EPA, 20:5n-3), DHA and AA by the human body from precursors available from vegetable oil and other sources [1]. The conversion rates are generally reported to be low [5-9] especially in infants [10-12]. In addition, iron, zinc, vitamin B6 and vitamin E are required for the synthesis of AA, EPA and DHA [13]. Most complementary foods are low in omega-3 fatty acids, and thus young children in low-income countries are at risk of low intakes [1, 4]. Fat is a critical component of breast milk, providing energy and key fatty acids for the development of the central nervous system of the infant. Among the principal fatty acids are DHA and AA [14]. However, the composition of fatty acids (FA) in breastmilk depend on the mother's diet and fat stores [2]. In the scientific literature, data on fatty acid intake and status among infants and women from low-income countries are scarce [1, 4, 15]. The objectives of this paper are to describe the fatty acid status of breast fed Nepali infants and their mothers using fatty acid RBC and breastmilk samples and to identify the main sources of fatty acids in the mother's diet.

Subjects and Methods

Study design and population

The selection criteria and details on field procedures have been described in detail elsewhere [16] . From January 2008 to February 2009, we enrolled 500 lactating women between 15-45 years of age and their infants below one year of age from the Bhaktapur municipality in Nepal. We used a two-stage cluster sampling procedure whereby 66 neighborhood streets called "toles" were randomly selected from the list of total 160. We listed all women living in these toles and women and infant pairs were randomly selected from this list [17]. The inclusion criteria for the study were that both mothers and children had no on-going infection (clinically assessed), resided in selected clusters, were willing to provide household information and consented to participate. A total of 1,101 eligible mother-infant pairs were identified during the inclusion period and 582 were approached for enrollment. Of these, 500 mother-child pairs were enrolled in the study and provided a blood sample. Due to limited resources, fatty acid composition of RBCs were analyzed in 303 infants of 321 children whom the mothers had agreed to be reenrolled in the study. To investigate associations between fatty acid status of infants and their mothers, fatty acid composition of RBC were analyzed from all lactating mothers with children from 4 months and below (n=72). Fatty acid composition of breastmilk samples (n=68) from the same mothers were also analyzed. These mothers were selected because their infants were most likely predominantly breast fed and had still RBC fatty acids reserves from prenatal life. All women provided written informed consent before the start of the study. The study had ethical clearance from the institutional review boards at the Institute of Medicine, Kathmandu, Nepal.

Breastmilk collection

Approximately 5 ml breastmilk (hind milk, non-fasting samples) were collected from each woman, and aliquots were stored at –80 degrees Celsius until analyzed. Sarstedt 20 ml plastic tubes were used for sampling and storage of the breastmilk samples. Women collected breast milk by manual expression and the breastmilk was collected on the same day and close in time to the collection of the urine samples. Infants were considered exclusively breast fed when they reportedly had only consumed breast milk from mother (or wet nurse or expressed breast milk) since birth and had not consumed any liquids or semi/solid foods except medicines.

Laboratory Procedures

Approximately 3 mL of whole blood was taken from the cubital vein using polypropylene tubes with lithium heparin (Sarstedt, Germany). The samples were then centrifuged (760x g, for 10 min, room temperature) and plasma was allocated into polypropylene vials (Eppendorf, Hinz, Germany). Samples were stored at -20°C at the field site laboratory until they were transported with an ice pack to the central laboratory in Kathmandu at the end of each day. There, samples were stored at -80°C until transport on dry ice to Norway. The FA composition of total RBC was determined by ultrafast gas chromatography (UFGC) (Thermo Electron Corporation, Massachusetts, USA), using a method developed by Araujo, Nguyen [18]. Briefly, 50 µl homogenized samples were mixed with boron trifluoride (BF3) and internal standard (19:0 methyl ester), followed by extraction with hexane. The fatty acid composition was calculated using a labdataprogram (Chromeleon 6.80, Dionex Corporation, California, USA), connected to the UFGC and identification ascertained by standard mixtures of methyl esters (Nu-Chek, Minnesota, USA). Limit of quantification was 10µg fatty acid/g samples (wet weight. w/w). The certified reference materials (CRM) CRM 162 (soy oil) and CRM 163 (pig fat) controlled the analytical quality of the method and systematic errors. The fatty acid composition of cooking oils was analyzed by gas liquid chromatography (GLC, Trace GC 2000, Termo) according to previously described method [19]. Total lipid content was extracted, filtered, evaporated, saponified and fatty acids were esterified. The methyl esters were separated using Auto-GC (Instrument-Teknikk AS, Norway), equipped with a 50m CP sil 88 (Chrompack) fused silica capillary column (id:0.32mm), using ‘‘cold on column’’ injection, temperature program (60 °C (25°C/min) to 160 (25°C/min) to 190 (25°C/min) to 220) and flame ionization detector. The fatty acid composition was calculated using a labdataprogram (Turbochrom Navigator, Version 6.1), connected to the GLC and identification as ascertained by standard mixtures of methyl esters (Nu-Chek, Elyian, USA). Nonadecanoic acid (19:0) methyl ester was used as internal standard. Limit of quantification (LOQ) was 10µg fatty acid/g sample (wet weight, w/w). The results are expressed as absolute and relative amounts. The omega 3 index was calculated as Sum %EPA + %DHA [20]. Delta-6 (D6D) and delta-5 desaturases (D5D) are key enzymes in the PUFA metabolism and they are encoded by FADS2 and FADS1. Estimates of D6D activity can be measured by using the product-to- precursor ratio of: 20:3n-6/18:2n-6 and D5D activity by 20:4n-6/20:3n-3 [21].

For verification of EPA in mustard and sesame oil, the samples consisting of mustard, sesame, cod liver, soy and sunflower oils were treated according to the protocol proposed by Araujo et al., 2016, [22] using LC-ESI-MS/MS. Triacylgrycerol (TAG) structures containing EPA

molecules were observed in mustard and sesame oil. DHA was not detected in any of these oils. The mass spectra for mustard and sesame oils confirmed the molecular weight of EPA at 302 m/z. Cod liver oil, as expected, contains a high number of TAG structures with omega-3 type fatty acids (EPA and DHA). TAG structures containing EPA or DHA molecules were not observed in soy or sunflower oils.

Dietary data collection

A detailed description of the dietary data collection and analysis has been published elsewhere [23]. Each woman participated in three quantitative 24-h recalls [24] and the recalls were obtained on three different days (including weekends, but excluding special holidays) (dietary recalls of the children were not collected). Because there is no standard food composition table available for Nepal, a food composition table was compiled for the present study which drew upon nutrient values for foods from WorldFood 2, the Nutritive Value of Indian Foods and, where necessary, from the Thai food composition table or the US food composition table. The 24-h dietary recalls were entered into nutrient analysis software designed for this study where it was possible to enter both dietary intake and personal recipes. The usual macronutrient intake distributions were calculated by the Multiple Source Method (MSM) [25, 26]. The dietary data provided information of the total amount of fat consumed by each woman and information of the most important sources to the fat intake, however the dietary recalls did not distinguish between different types of cooking oil consumed by each women.

All mothers were asked at what age they introduced soft and semi-solid food to their infants.

Children were weighed on a UNICEF electronic pediatric scale (SECA, Germany) and length was measured using a locally made wooden board with an accuracy of ± 0.1 cm .The Z-scores for height-for-age (HAZ), weight-for-length (WLZ) and weight-for-age (WAZ) were calculated using WHO growth reference standard from 2006 [27]. Children were classified as stunted, wasted, or underweight if their HAZ, WLZ, or WAZ was <-2 SD, respectively. For women, BMI was calculated as weight/ (height) ^{2} (kg/m^2). BMI <18.5 kg/m^2 was considered as underweight, 18.5 kg/m^2 <BMI ≥ 25 kg/m^2 as normal weight and BMI ≥ 25 kg/m^2 as overweight.

Statistical analysis

Data were entered and analysed using SPSS version 22 (SPSS Inc., Chicago) and STATA version 13 (StataCorp., College Station,TX, USA). Normally distributed data was expressed as mean (±SD). Continuous variables were compared by Independent sample t-test, two-tailed

tests with a 5% significance level were used for all analyses. The correlations between infants' DHA, EPA- and AA- concentration and mothers' DHA, EPA- and AA- concentration were investigated by calculating Spearman's *r*. DHA and AA were used as dependent variables in multiple linear regression analyses. A theory-based approach was used to select candidate variables for inclusion in the models. All covariates showing a linear association ($p<0.10$) in the crude regression models were included in a preliminary multiple regression model. The following variables believed to influence DHA and AA were included in the initial crude models; gender, age, exclusively breastfeeding, birth weight, birth order, WHZ, HAZ, mothers total intake of fat in grams, BMI mother, number of children, mothers literacy, working mother. A manual stepwise regression was performed, where excluded variables were reintroduced and those that were still significantly associated in this model ($p<0.10$) were retained in the final model [28]. Analysis of the residuals was performed in order to examine the fit of the model. The dose-response graphs were constructed using kernel-weighted local polynomial regression in Stata 13.

Results

Background characteristics

The mean (SD) age of the infants was 7.1 (1.9) months and for the women 26.2 (4.1) years (Table 1). Forty-two percent of the infants were exclusively breast fed to 3 months and 18 % to 6 months. The prevalence of underweight and stunting was 5 and 9 %, respectively. The 197 of the 500 infants who were not included in this analysis, were slightly younger, had slightly lower birth weight and a higher proportion of the mother were illiterate, which may reflect that this labile population of Bhaktapur has lower socioeconomic status (data not shown).

Fatty acid concentration.

The mean concentration of DHA, EPA and AA for all infants were 116, 8.3 and 311 µg/g, respectively (Table 2). DHA was the main n-3 fatty acid in both mothers and infants, as AA was the major n-6 fatty acid. The concentration of total *n*-6 PUFA was three and four times higher than total *n*-3 PUFA for children and women, respectively. The concentration of all fatty acids were higher in children, compared to mothers, except Total n-6 PUFA and Linoleic acid (LA), where no differences were found The n-6/n-3 ratio was higher in women (4.2) than in children (3.2), whereas the omega-3 index was lower in women (4.4) than in children (5.1). In breastmilk, the mean concentration of DHA, EPA and AA was 49, 43 and 231 µg/g, respectively, the n-6/n-3 ratio was 6.6 and the omega-3 index was 0.2. The values for D6D in mothers and children were 0.15 (0.03) and 0.13 (0.03), respectively and for D5D the values were 8.1 (1.6) for the mothers and 9.8 (1.9) for the children.

Correlations of fatty acid composition between infants, mothers and milk

A weak correlation was found between RBC-DHA in children and mothers, r=0.33, p=0.005, no significant correlations were observed between RBC-DHA in infants and DHA in breastmilk, nor between RBC-DHA in mothers and DHA in breastmilk. A weak correlation was found between RBC-EPA in mothers and EPA in breastmilk, r=0.23, p=0.06. No significant correlations were found for RBC-AA in infants, mother or breastmilk.

Multiple regression models were used to identify determinants of DHA and AA status in infants (Table 3). In the final models, HAZ was positively associated with infants´DHA and AA status. In contrast with findings in figure 1, age was only associated with infants´AA, but not DHA status."

The women's dietary intake of fat

The mean (SD) energy intake among the women was 2023 ± 312 kcal, the mean (SD) fat intake was 30 ± 9 g/d and the energy intake derived from fat was 13%.. Cooking oil (mustard, soybean, sesame and sunflower oil) contributed more than half of the fat intake though we could not distinguish between the relative contributions of each of these oils (52%) (Table 4). Buffalo milk consumed with tea or by itself also made a considerable contribution (12%). Gee, or unclarified butter made from buffalo milk, contributed about 3% of fat intake.

Fatty acid composition of the most important of dietary sources of fat .
Soybean and sunflower oils contain high concentrations of LA, whereas mustard and sesame oils are the main source of ALA (Table 5). Noteworthy, both mustard oil and sesame oil contain small amounts of EPA. Mustard oil have high concentration of erucic acid (22:1n-9), medium concentrations are seen in sesame oil, and low concentrations in both soybean and sunflower oils were found. Buffalo milk contains 0.07 mg/g of ALA and 0.4 mg/g of LA and, low concentration of erucic acid and AA, and no EPA was detected. The fatty acid composition of the different types of local ghee varied considerably. However, they are a source of LA, ALA, further AA, EPA and erucic acid was found in some of them. None of the cooking oils and ghee contained more than 1 mg/g AA and DHA was not detected in any of the analyzed food items.

Discussion

This study was conducted in a population with relatively low intake of fish. It was therefore somewhat surprising to find that the randomly selected infants had RBC-DHA and RBC-AA comparable to European children. Also their mothers had relatively high concentrations of these fatty acids. According to FAO/WHO [29] levels on adequate intake (AI) for breastmilk, concentrations of AA were found to be adequate, whereas the concentrations of DHA were low.

The RBC-DHA and RBC-AA observed in children were comparable to those in a randomized controlled trial of healthy German infants, where complementary foods either enriched with ALA-rich rapeseed oil, LA-rich corn oil or salmon (DHA), were given from the age of 4 months until age of 10 months [30]. In that trial, the RBC-DHA in the two groups with

rapeseed oil and corn oil declined from 5.9% to 4.5%, that was even lower than observed in our study (4.9%). In contrast, the levels observed in infants who received salmon maintained their initial RBC-DHA level of 6.3% at the end of the experiment. In another sample of Norwegian infants (3 month of age), observed concentrations of RBC-DHA averaged 181 µg/g [31], which is considerably higher than what we found in our study (mean value for all infants was 116 µg/g). In a study from Nigeria [32], the RBC-AA was 12.8% and RBC-DHA was 3.40%, which is comparable to our findings of 13.2% AA and 4.9% DHA.

. The relatively high levels of DHA and EPA in the infants in our population, suggests that infants had a reasonable intake of these fatty acids through a dietary source. Fish is rarely fed to infants in Bhaktapur, and was only consumed by four mothers in our survey, suggesting that intake of natural preformed sources of DHA is not a likely explanation for the unexpected high DHA levels of infants. Our study did not collect extensive data on complementary feeding, but did find that consumption of commercial complementary foods was reported by 11% of mothers, while 40% reported giving their infant a homemade porridge based on maize, rice and cooking oil (Lito). A recent study, also set in Bhaktapur, reported that 25% of children 6-23 months had consumed commercial complementary food, which suggests that we may have underestimated the use of commercial complementary foods [33] or that the use of commercial complementary foods has increased during the last decade. Another possible explanation relates to the conversion of ALA into EPA, which is generally assumed to be low, about 9% [34] and the capacity of the fetus to synthesize AA and DHA is limited [35]. However, genetic variation in the *FADS gene cluster* and *ELOVL gene family* can alter the efficiency or expression of the elongases responsible for this conversion. Gene-environment interactions involving the *ELOVL* family and *FADS* gene cluster in combination with the lack of preformed DHA in the diet may have led to up-regulation of this conversion [36-38].

Low DHA concentration in breastmilk may be a risk factor for development delays in children [39]. AA and DHA are found in relative high amounts in human tissue, including brain, during pregnancy and infancy, and these fatty acids are transported across the placenta to the developing fetus [40]. Breast-fed infants receive these fatty acids from human milk; hence, the fatty acid composition of the breastmilk is crucial for optimal growth and development. In our study, the content of LA in breastmilk was approximately ten times higher than the content of ALA, in concordance with the lipid profile of the cooking oils consumed by the Nepali women. However, the two fatty acids of greatest public health concern in breastmilk are AA and DHA [35]. FAO/WHO have set AI for AA in breastmilk

between 0.2% and 0.4% of fatty acids [29]. The mean AA in breastmilk in the present study was 0.6% of the fatty acids, and even the lowest measured concentration (0.4%) was within the AI. The mean AA in breastmilk in the present study was also higher than reported in a review of 65 studies on fatty acid composition of breastmilk (0.47%) of women from both low- and middle-income [41]. In regard to DHA, a level of 1% has been suggested as optimal in western breastmilk [42]. In our study, DHA in breast milk was 0.1%, and very low compared to a mean DHA of 0.32% (0.06-1.4) reported in the review above. Only results from Pakistan (0.06%) and South Africa (0.1%), were comparable to our findings; results from the other countries suggested higher DHA (0.15-0.91% fatty acids) [41]. An earlier study on fatty acid composition of breastmilk from Nepal, reported a 0.21% of DHA [43], which is higher than reported in our study. Based on dietary data of the lactating women in the present study, we know that intake of marine foods were limited and cooking oils with no DHA constituted 52% of the fat intake. This is probably the explanation for the low concentration of DHA in breastmilk.

Noteworthy, in our study, the level of DPA (0.2%) in breastmilk was higher than DHA (0.1%). In the INFAT study [44], the level of DPA and DHA was 0.17% and 0.28%, respectively in breastmilk from healthy German women. Whereas for women who consumed 1020 mg DHA + 180 mg EPA per day from 15th week of gestation until four months postpartum, the levels were 0.23% for DPA and 1,34% for DHA. In breastmilk from a community in Bolivia and from USA, the level of DPA was 0.40% and 0.14%, respectively, and for DHA 0.69% and 0.16%, respectively [45]. In a group of women from Nigeria, the level of DPA and DHA was reported to be 0.08% and 0.20% [32]. The higher level of DPA than DHA and a very low EPA level among the women in the presents study may be explained by desaturation and elongation activity.

In our study we found a significant correlation between RBC-DHA in infants and in mothers, in accordance with two studies in Tanzania [46, 47]. In a study from Nepal [48] and a study from Nigeria [32] a positive association between fatty acids RBC in women and fatty acids in breastmilk was found, this was also reported for a German population, although not very strong [44]. In contrast to our study from Nepal and a study from Sri Lanka were no such association were found [49]. The explanation for the lack of association between breastmilk fatty acid and RBC in mother in our study may be that the level of milk-DHA was low, due to low intake of DHA, restricted desaturation of ALA to DHA, together with depleted levels in adipose tissue. In a study from Nigeria a significant correlation between fatty acids in breastmilk and fatty acids RBC in their infants was found [32]. However, in German infants

[44] no association of AA, EPA and DHA in breast milk and the respective fatty acids in RBC in infant at four and twelve months after birth was reported, in accordance with our findings. The lack of association between fatty acids in infants and breastmilk may be explained by an increased desaturation of ALA to DHA in infants, as argued above. In addition, due to the young age of the participating infants some of them might have fat reserves from pre-natal life.

A suboptimal intake of omega-3 fatty acids, and DHA in particular, during pregnancy, lactation and early life, may have significant impact on infant growth and development [1, 50]. In our study, children with low HAZ had a lower RBC-DHA and RBC-AA, which may suggest a potential link between suboptimal fatty acid status and linear growth faltering, though the cross-sectional design of our study and potential for unmeasured confounding limits our ability to infer causality. A pathway analysis undertaken as part of a trial of micronutrient supplements and fortified fat-based spreads suggested that ALA in particular was associated with greater growth [51] indicating that suboptimal intake of these fatty acids may influence linear growth [52]. In a study from China, an association between intake of PUFA and early childhood growth among preschool children was found, suggesting that growth could be related to n-6 fatty acid intakes [53].

The mothers had relatively high level of DHA despite low presumed dietary intake of preformed DHA and ALA. A study in Chilean pregnant women found that the dietary intake was low in long chain n-3 fatty acids [54]. However, the mean level of RBC-DHA (3.6%) was lower than in the Nepali women (4.5%), whereas it was opposite for RBC-EPA, 1.6% (Chile) and 0.5% (Nepal). The Chilean women had an omega-3 index of 5.2% compared to 4.5% in the Nepal women. One explanation for the higher level of DHA in the Nepali women may be that they had a considerably lower fat intake, about one third of the Chilean women. ALA and LA are essential fatty acids and can to some extent be desaturated and elongated to DHA and AA, respectively [30] and low-fat diets have been associated with significantly higher levels of DHA in intervention trials [55, 56]. In addition, the Chilean women had a relatively high intake of LA 4.4 g/day (median), which can adversely affect the EPA and DHA formation [47]. Comparing levels of DHA- from Chinese women (5.6%), Belgium women (4.8%) and USA women (4.7%) [54], Chilean women had the lowest level of DHA , and the Nepali women had the second lowest. In Norwegian pregnant women [57], level of RBC-DHA (6%) was slightly higher than reported for the Chinese women (5.6%). The infant level of RBC-DHA (4.9%) in this study was higher than their mothers (3.9%). One possible explanation may be biomagnification of DHA, a process that has been found to occur when maternal RBC-DHA is lower than 5.6% [46]. In a study of three ethnic groups in Tanzania with no, medium and high fish intake, biomagnification occurred up to a maternal level of DHA-RBC of 8% [47]. This hypothesis is also supported by high ratio of D5D found in the present study, indicating elevated activity of delta-5 desaturase in Nepalese women and infants. The D6D in the Nepalese mothers and children are similar to what have been reported in previous studies, whereas the levels for D5D are twice as high, although not same tissue and population [21,

37, 58, 59]. Values for erythrocytes in pregnant Norwegian women [60], were 0.13 for D6D and 6.7 for D5D. In erythrocytes from German infants [44], the values were 0.18 (16 weeks postpartum) and 0.12 (1 year postpartum) for D6D and the D5D values were 8.4 (16 weeks postpartum) and 7.5 (1 year postpartum).

The total intake of dietary fat of the mothers was low (13% of the total energy intake) compared to the EFSA recommendations of an energy percent of 20-35 % from fat [61]. Low dietary intake of fat increases the risk of suboptimal intake of essential fatty acids [62]. The main source of fat in the diet of the Nepali women was cooking oils (sunflower, soybean, sesame and mustard). The relatively high concentration of LA in the cooking oils ensured a substantial intake of this essential fatty acid in their diet, even though we could not distinguish between the relative contributions of each of these oils. For ALA, the concentration in cooking oil was considerably lower, giving a much lower total intake of ALA. Cooking oils and ghee had very low concentrations of AA; no item provided more than 1 mg/g and DHA was not detected in any of the analyzed food items. Even though EPA has previously been found in mustard oil [63] and other vegetable oils [64], it was unexpected to detect EPA in mustard oil and sesame oil. As far as we know, there are no reports on delta-6 or delta-5 desaturase activity in these oils, and we cannot exclude the possibility that the oils were not pure. The oils contained high amounts of erucic acid, which has been associated with myocardial lipidosis in a number of species and heart lesions in rat [65]. EU has established a maximum concentration of 5% erucic acid in edible oils and fat [66]. Studying such consequences was not part of our study objectives and remains a research question for further exploration. European Food Safety Authority (EFSA), has determined the AI to be 250 mg EPA + DHA/day and to increase by 100-200 mg DHA during pregnancy and lactation [61]. In our study, the lactating women are far from reaching the recommended intake of the long chain n-3 fatty acids from their diet.

This is one of the first studies of fatty acid status in South Asia and the first from this peri-urban population in Nepal. In addition to the relatively large sample size of the child sample, one of the strengths of our study is that we were able to collect and analyze data on blood samples of both mothers and children as well as breastmilk to explore associations between these measures.. Our ability to explore associations between mother and child status may have been limited by the sample size. . Other limitations of our study were that our maternal dietary recalls did not distinguish between different types of cooking oil and that we did not collect

extensive information on complementary foods consumed by infants. In addition, we did not have information on genotyping. Further, there are no reference values for PUFA and results are compared with findings from other studies which also have used other biomarkers at other ages which may limit the comparability. Thus, we cannot conclude on adequacy of fatty acid status.

In conclusion, the children and mothers have RBC DHA levels comparable to European children and mothers despite low concentration of DHA in the diet and breast milk. Some of the vegetable oils commonly used in food preparation contained EPA and may be a significant dietary source. The unexpected high RBC-DHA levels may reflect a higher conversion rate of ALA and LA in this population.

Conflicts of Interest: The authors declare no conflict of interest.

Compliance with Ethical Standards.

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Table 1 Background characteristics of the Nepali infants (n=303) and mothers (n=72)

Characteristics	Values
Infants	
Age infant, months	7.1 (2.9)
Birth weight, gr	2672.7 (880)
Newborn with <2500 g of birth weight, % (n)	21 (63)
Home delivery, % (n)	3 (10)
Initiation of breastfeeding within one hour, % (n)	80 (242)
Colostrum given, % (n)	94 (283)
Exclusively breast fed for 3 months of age, % (n)*	43 (112)
Exclusively breast fed for 6 months of age, % (n) #	17 (49)
Age complementary feeding introduced, m	2.7 (2.3)
Underweight (<-2 z score weight for age), % (n)	5 (16)
Stunted (<-2 z score length for age), % (n)	9 (27)
Mothers	
Age mother, y	26.2 (4.1)
Mother with <18.5 BMI (kg/m^2), % (n)	3 (10)
Mother with >25 BMI (kg/m^2), % (n)	18.5 (56)
Mother with two or more parity, % (n)	84.5 (256)
Illiterate, % (n)	11.9 (36)
Working outside home, % (n)	36.3 (110)

Mean (SD)/ % (n)
*n=260, 43 women were still exclusively breastfeeding when the question was asked
#n=282, 21 women were still exclusively breastfeeding when the question was asked

Table 2 Fatty acid composition of red blood cells (µg/ml and %) in women (n=72), breastmilk (68) and red blood cells in infants (n=303).

	Women (n=72)		Breast milk (n=68)		Infants (n=303)		p-values#
	(µg/ml)	%	(µg/ml)	%	(µg/ml)	%	
Total SAFA	840 (113)	40	19292 (7681)	50	948 (99)	41	0.001
Total MUFA	478 (105)	23	11986 (4886)	31	449 (76)	19	0.02
Total PUFA	752 (94)	36	6874 (2982)	18	906 (158)	38	0.001
Total *n*-6 PUFA	605 (75)	29	5864 (2577)	16	685 (128)	29	0.10
Total *n*-3 PUFA	147 (24)	7.0	991 (573)	2.5	222 (44)	9.4	0.001
18:2 *n*-6 (LA)	231(45)	11.1	5303 (2398)	14.0	269 (76)	11.3	0.66
18:3 *n*-3 (ALA)	10 (1)	0.3	568 (313)	1.5	5.4 (5)	0.2	0.01
20:4 *n*-6 (AA)	268 (35)	12.9	231 (84)	0.6	311 (59)	13.2	0.01
20:5 *n*-3 (EPA)	12 (4)	0.5	43 (20)	0.1	8.3 (4)	0.3	0.001
22:6 *n*-3 (DHA)	81 (15)	3.9	49 (20)	0.1	116 (22)	4.9	0.001
22:5 *n-3* (DPA)	40 (9)	1.9	60 (25)	0.2	37 (11)	1.6	0.001
n-6/n-3 PUFA *Ratio*	4.2 (0.5)		6.6 (2.1)		3.2 (0.6)		0.001
Sum fatty acids	2089 (264)		38576 (14539)		2345 (293)		0.001
Omega 3 index %*		4.4		0.2		5.2	

Mean ± SD (absolute values)
*Sum %EPA + %DHA (% of sum of total fatty acids)

p-values between women and infants tested with independent samples t-test

Table 3 Determinants for DHA (22:6, n-3) and AA (20:4, n-6) in Nepali infants (n=303)

Dependent variables	Predictor variables	Unadjusted coefficient (95% CI)	*p*	Adjusted coefficient (95% CI)	*p*	Stand Beta
DHA[d], *mg/ml*	Constant			0.108 (0.101, 0.114)	*<0.001*	
	Age[b]	0.1 (0.0, 0.2)	*<0.001*	0.1 (0.0, 0.2)	*0.14*	0.01
	Ex.BF[b]	0.1 (0.0, 0.3)	*0.01*	0.1 (0.0, 0.2)	*0.05*	0.12
	HAZ[c]	0.1 (-0.1, 0.4)	*0.22*	0.2 (0.0, 0.5)	***0.04***	0.12
	R^2				0.09	
AA[e], *mg/ml*	Constant			0.30 (0.22, 0.31)	*<0.001*	
	Age	0.2 (0.1, 0.4)	*0.03*	0.3 (0.0, 0.5)	***0.02***	0.15
	Ex.BF	0.3 (0.2, 0.6)	*0.04*	0.3 (-1.0,0.5)	*0.11*	0.10
	HAZ	-2.0 (-4.5, -0.3)	*0.02*	0.3 (0.0, 0.5)	***0.002***	0.19
	R^2				0.06	

[a] Continues variable in months
[b] height/length for age, ccontinues variable
[c] 3 outliers for DHA, n=300
[d] 6 outliers for AA, n=297

Table 4 An overview of the food items and food groups in percent that contributed most to the fat intake in in the lactating mothers (n=72)

Food item	Food groups	%
Cooking oil*	Fat and edible oils	51.9
Milk buffalo	Milk and milk products	12.1
Beaten rice	Cereal grains and products	3.4
Chicken meat	Meat and poultry	3.1
Ghee buffalo[#]	Fat and edible oils	2.8
Rice cooked	Cereal grains and products	2.6
Egg hen	Meat and poultry	2.6
Biscuits, sweet	Snacks	2.0
Curds	Milk and milk products	1.9

*The most common used cooking oils are mustard, soybean, sunflower and sesame- oil.
[#] Butter made from buffalo milk

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655 **Table 5 Fatty acid composition of some important oils, buffalo milk and Ghee (buffalo)* mg/g**

	Sunflower		Soybean		Mustard		Sesame		Milk		Ghee	
	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%	mg/g	%
SAFA[#]	113	12	119	13	61.2	7	125	13	15.7	61	432	57
MUFA[#]	243	26	229	27	636	68	326	34	8.6	33	264	34
PUFA [#]	576	62	534	60	230	25	479	51	0.8	3	37	4.6
18:2 *n*-6	569	61	515	58	149	16	435	46	0.4	1.5	27	3.3
18:3 *n*-3	5.5	0.6	12.0	1.5	73.3	7.8	34.0	3.6	0.07	0.3	2.4	0.3
20:4 *n*-6	<0,01	<0.1	<0,01	<0.1	0.1	<0.1	<0,01	<0.1	0.03	<0.1	0.3	<0.1
20:5 *n*-3	<0,01	<0.1	<0,01	<0.1	4.1	0.5	1.2	0.2	<0,01	<0.1	0.5	0.1
22:6 *n*-3	<0,01	<0.1	<0,01	<0.1	<0,01	<0.1	<0,01	<0.1	<0,01	<0.1	<0,01	<0.1
22:1 n-9	4.3	0.5	4.1	0.5	263	28	75	12.8	0.07	<0.1	0.3	0.2

* Two samples of every oil, buffalo milk and Ghee were analyzed
[#] Total

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