Maternal DHA Equilibrium during Pregnancy and Lactation Is Reached at an Erythrocyte DHA Content of 8 g/100 g Fatty Acids\textsuperscript{1,2}

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Abstract

Low long-chain PUFA (LC-PUFA, or LCP\textsuperscript{7}) consumption relates to suboptimal neurodevelopment, coronary artery disease, and (postpartum [PP]) depression. Maternal-to-infant LCP transport during pregnancy and lactation is at the expense of maternal status, a process known as biomagnification. Despite biomagnification, maternal and infant LCP status generally declines during lactation. To assess the \textsuperscript{1}) turning point of biomagnification (level from which maternal [m]LCP status exceeds infant [i]LCP status); \textsuperscript{2}) LCP equilibrium (steady-state-level from which mRBC-LCP stop declining during lactation); \textsuperscript{3}) corresponding iLCP-status; and \textsuperscript{4}) the relationship between RBC-DHA and RBC-arachidonic acid (AA), we measured RBC-fatty acids in 193 Tanzanian mother-infant pairs with no, intermediate (2–3 times/wk), and high (4–5 times/wk) freshwater fish consumption at delivery and after 3 mo of exclusive breast-feeding. At 3 mo, mRBC-DHA was lower than the corresponding iRBC-DHA up to a mRBC-DHA of 7.9 g%. mRBC-DHA equilibrium, with equivalent mRBC-DHA at both delivery and at 3 mo PP, occurred at 8.1 g%. This mRBC-DHA equilibrium of 8.1 g% corresponded with an iRBC-DHA of 7.1–7.2 g% at delivery that increased to 8.0 g% at 3 mo. We found between-group differences in mRBC-AA; however, no differences in iRBC-AA were observed at delivery or 3 mo. Relations between RBC-DHA and RBC-AA were bell-shaped. We conclude that, at steady-state LCP intakes during lactation: \textsuperscript{1}) biomagnification occurs up to 8 g% mRBC-DHA; \textsuperscript{2}) mRBC-DHA equilibrium is reached at 8 g%; \textsuperscript{3}) mRBC-DHA equilibrium corresponds with an iRBC-DHA of 7 g% at delivery and 8 g% after 3 mo; \textsuperscript{4}) unlike RBC-DHA, mRBC-AA and iRBC-AA are independently regulated in these populations; and \textsuperscript{5}) bell-shaped RBC-DHA vs. RBC-AA-relationships might support uniform iRBC-AA. A (maternal) RBC-DHA of 8 g% might be optimal for infant neurodevelopment and adult cardiovascular disease incidence. J. Nutr. 141: 418–427, 2011.

Introduction

The long-chain PUFA (LC-PUFA, or LCP\textsuperscript{7}) DHA, EPA, and arachidonic acid (AA) are structural components of membrane phospholipids (PL), modulators of gene expression, and precursors of eicosanoids (AA, EPA, DHA), and (neuro)protectins (DHA) \textsuperscript{(1)}. DHA and AA are notably abundant in the central nervous system (CNS) and play important roles in fetal and infant neurodevelopment (2). A balance between EPA and DHA on the one hand, and AA on the other, is important because of its implication in e.g. coagulation and inflammation (1). Horrobin et al. (3) suggested that collaboration between EPA and AA plays a key role in the beneficial effects of low-dose EPA in psychiatric disease. Low DHA in the CNS has been suggested to decrease DHA turnover, with reciprocal increase in AA turnover (4). High DHA intakes reduce AA in erythrocytes (RBC) (5) and possibly in brain (6). Low (n-3) LCP status is intimately related to cardiovascular and psychiatric diseases in adults (7). Most populations living in Western countries are characterized by low intakes of (n-3) LCP (especially EPA and DHA), which contrasts with the derivation of our ancestors from a land-water ecosystem with abundantly available (n-3) LCP and (n-6) LCP (especially AA) (8).

With advancing gestation, the pregnant mother increasingly transfers LCP, notably DHA, to the developing infant. Compared with its mother, infant plasma lipids and RBC in the second half of pregnancy contain higher relative amounts of LCP, which has been coined biomagnification (9). We have recently shown that biomagnification of DHA might actually reflect low maternal (n-3) LCP status, because infants born in a

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\textsuperscript{7} Abbreviations used: AA, arachidonic acid; FA, fatty acid, LCP, long-chain PUFA (LC-PUFA); PL, phospholipid; PP, postpartum; RCT, randomized controlled trial.
population with lifetime high freshwater fish intakes have lower cord blood RBC-DHA compared with their mothers. We found that this ‘bioattenuation’ occurs from a maternal RBC-DHA at term of ~6 g%, which corresponds with a maternal RBC-DHA of ~6 g% in early pregnancy (M. Luxwolda, R. Kuipers, W. Sango, G. Kwesigabo, D. Dijck-Brouwer, F. Muskiet, unpublished data). After birth, breast-fed infants in Western populations show consistent decreases in RBC-DHA (10,11). This might reflect low maternal (n-3) LCP status, analogous to the intrauterine period, because infants born to mothers with lifetime high intakes of freshwater fish exhibit postpartum (PP) RBC-DHA increases (M. Luxwolda, R. Kuipers, W. Sango, G. Kwesigabo, D. Dijck-Brouwer, F. Muskiet, unpublished data).

Although the transfer of nutrients via lactation is generally considered to be beneficial for the infant, it may diminish maternal stores, resulting in the so-called maternal depletion syndrome (12). Maternal LCP depletion, which affects DHA more than AA, may be most pronounced with longer gestation, short birth intervals, increasing parity, and multiple pregnancy (13,14). Whereas AA in maternal plasma PL and RBC-AA increase to prepregnancy values after delivery, there is a consistent PP decrease of maternal plasma PL- and RBC-DHA in lactating compared with nonlactating women (15). Low intakes of (n-3) LCP during pregnancy were reported to result in slightly shorter gestation, marginally lower birth weight and increased risk of preterm delivery (16). Low maternal DHA status in populations with low seafood consumption has also been associated with a higher incidence of PP depression (17). The causality is, however, uncertain, because randomized controlled trials (RCT) with DHA in pregnancy and lactation have been inconclusive (18,19). For instance, in a placebo-controlled trial, supplementation with 200 mg/d DHA for the first 4 mo after delivery prevented PP decline in plasma PL-DHA but did not influence PP depressive symptoms (19). A recent small trial showed beneficial effects of (n-3) LCP supplementation on depression during pregnancy (20). Finally, a declining maternal DHA status during pregnancy was suggested to be involved in compromised maternal selective attention, a key component of cognition (21).

We investigated the transplacental LCP gradient and the courses of maternal and infant RBC-LCP during lactation in 3 rural African tribes with constant, lifetime low, intermediate, and high intakes of tropical freshwater fish. We were particularly interested to determine: 1) the point where biomagnification turns into bioattenuation, i.e. the level from which the maternal LCP status exceeds the infant LCP status; 2) the DHA status at which the mother reaches a PP RBC-DHA equilibrium, i.e. the steady-state maternal RBC-DHA level at delivery that suffices to prevent a decline in the maternal DHA status during subsequent lactation; 3) the PP course of infant RBC-DHA that corresponds with maternal PP DHA equilibrium; and 4) the relation between RBC-DHA and RBC-AA at low and high fish intakes.

### Participants and Methods

**Study design.** We studied the transplacental LCP gradient at delivery and the course of the maternal and infant LCP status during exclusive lactation. For this, we selected 3 ethnic groups in Tanzania with different intakes of (n-3) LCP from local fish, i.e. the Maasai (no or low fish intake), participants from the Pare Mountains (intermediate fish intake), and participants from Sengerema (high fish intake). Each of these groups was considered homogeneous with respect to ethnicity/tribe and their lifetime dietary habits. The data for the transplacental gradient were derived from 3 groups of healthy and well-nourished mothers who delivered apparently healthy infants at term (37–42 wk). Data on the course of the LCP status in mothers and infants during exclusive lactation were derived from the comparisons of the LCP status of these mothers/infants with those of counterparts at 3 mo PP. The latter were healthy, well nourished, and had delivered an apparently healthy infant at term (37–42 wk of gestation) infant 10–18 wk prior to their visit to the local hospital or dispensary for the follow-up of their infant in the pediatric department. All women gave their informed consent. The study was approved by the National Institute for Medical Research in Dar-es-Salaam (NIMR/HQ/R.8a/Vol. IX/800, dated April 8, 2009) and was in agreement with the Helsinki declaration of 1975 as revised in 2000.

### Table 1

**Anthropometrics of mothers and infants at delivery and at 3 mo PP**

<table>
<thead>
<tr>
<th></th>
<th>Massai</th>
<th>Pare</th>
<th>Sengerema</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age, y</td>
<td>23 ± 4 (8)</td>
<td>25 ± 7 (31)</td>
<td>24 ± 7 (34)</td>
</tr>
<tr>
<td>PP weight, kg</td>
<td>53.0 ± 5.8 (8)</td>
<td>57.3 ± 8.3 (23)</td>
<td>54.4 ± 10.3 (31)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.59 ± 0.06² (8)</td>
<td>1.54 ± 0.04⁴ (27)</td>
<td>1.56 ± 0.06³ (31)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.9 ± 2.0 (8)</td>
<td>23.9 ± 2.0 (21)</td>
<td>22.4 ± 3.3 (31)</td>
</tr>
<tr>
<td>Gravida, n</td>
<td>3 ± 1 (8)</td>
<td>3 ± 2 (31)</td>
<td>3 ± 3 (34)</td>
</tr>
<tr>
<td>Para, n</td>
<td>2 ± 1 (8)</td>
<td>2 ± 2 (31)</td>
<td>2 ± 3 (34)</td>
</tr>
<tr>
<td>Gestational age at birth, wk</td>
<td>40.0 ± 0 (1)</td>
<td>39.9 ± 1.5 (17)</td>
<td>38.9 ± 1.8 (20)</td>
</tr>
<tr>
<td>Fish intake, times/wk</td>
<td>0 ± 0 (8)</td>
<td>3 ± 2º (17)</td>
<td>5 ± 2³ (30)</td>
</tr>
<tr>
<td>Infant birth weight, g</td>
<td>3050 ± 600 (8)</td>
<td>3115 ± 535 (26)</td>
<td>2947 ± 751 (34)</td>
</tr>
<tr>
<td>Gender, % male</td>
<td>38 (8)</td>
<td>45 (29)</td>
<td>58 (36)</td>
</tr>
</tbody>
</table>

| **3 mo PP**    |             |             |             |
| Maternal age, y| 24 ± 4 (9)  | 24 ± 4 (40) | 24 ± 6 (61) |
| Weight, kg     | 52.4 ± 3 (9) | 52.2 ± 11 (40) | 54.6 ± 10 (61) |
| Height, m      | 1.60 ± 0.03² (9) | 1.55 ± 0.07³ (40) | 1.56 ± 0.06³ (61) |
| BMI, kg/m²     | 20.5 ± 1.6¹ (9) | 21.6 ± 4.0 (40)³ | 22.1 ± 3.0³ (60) |
| Para, n        | 3 ± 1 (9)   | 2 ± 1 (40)  | 3 ± 2 (61)  |
| Fish intake, times/wk | 0 ± 0.7³ (8) | 3 ± 2³ (40) | 4 ± 2³ (61) |
| Infant age, wk | 16 ± 4 (9)  | 14 ± 3 (40) | 13 ± 2 (61) |
| Gender, % male | 67 (9)      | 47 (40)     | 55 (61)     |

*Values are mean ± SD (n). Means in a row with superscripts without a common letter differ, P < 0.05.*
Lake is located 30 km from their villages. The Sengerema group included Bantu women from the fishery families living on the southern shore of Lake Victoria who attended to the hospital of Sengerema. Ugali, cassava root, and plantain are staple foods, but consumption of fish is markedly more regular in the Lake Victoria area than for inhabitants of the Pare area. Smoking and alcohol consumption are very uncommon within these communities, especially among women. Importantly, included participants had neither the possibility to (Pare and Sengerena) nor major interest in (Maasai) deviation from their cultural habits, including their diets. We observed, and local doctors, nurses, and participating women confirmed, that neither pregnancy nor lactation was associated with any change in dietary habits in any of the investigated groups. The average dietary intakes of mothers at delivery as well as at 3 mo PP were therefore likely to be representative for the lifetime dietary habits of their respective ethnic group/trbe as a whole. Data on age, parity, fish consumption, and duration of lactation were obtained from the medical records or by interviews in Kiswahili. Gestational ages were checked by the last known menstrual period and fundal height. After delivery, all infants were routinely checked for signs of prematurity.

**Samples and analyses.** About 4 mL EDTA-anticoagulated venous blood samples (7.2 mg of sprayed K2EDTA; in 4 mL tubes; BD Vacutainer) were collected from the mothers at delivery and after 3 mo and from the umbilical vein at delivery. An ~250 mL EDTA-anticoagulated blood sample (250 mL pediatric MiniCollect K2EDTA; Greiner Bio-one) was taken from the 3-mo-old infants via a heel prick. Samples were stored at 4°C in the dark and processed within 2 h after collection. RBC were separated from plasma by centrifugation and washed 3 times with 0.9% NaCl. After washing, aliquots of 200 μL of RBC suspension (4-mL tubes) or the entire RBC suspension (250-mL tubes) were pipetted into a Teflon-sealable Sovirel tube containing 2 mL of methanol 6 mol/L HCl (5:1, v:v), 1 mg BHT (antioxidant), and 50 μL of a ready-to-transmethyle mixture, fatty acids (FA) are stable transported at room temperature to the University Medical Center.

### Table 2: Maternal and infant erythrocyte FA at delivery and after 3 mo lactation by mothers’ fish intake

<table>
<thead>
<tr>
<th>Mothers</th>
<th>Delivery</th>
<th>3 mo PP</th>
<th>g/100 g FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mothers, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAFA</td>
<td>6</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>MUFA</td>
<td>21.4±1.6 (9.0–24.0)</td>
<td>17.1±1.6 (15.5–19.5)</td>
<td>20.3±1.6 (18.0–24.6)</td>
</tr>
<tr>
<td>PUFAs</td>
<td>34.6±4.1 (24.1–36.5)</td>
<td>38.7±4.1 (37.9–39.7)</td>
<td>34.8±4.1 (27.6–39.3)</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.23±0.06 (0.11–0.28)</td>
<td>0.27±0.06 (0.20–0.34)</td>
<td>0.14±0.06 (0.02–0.28)</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.18±0.03 (0.03–0.07)</td>
<td>0.43±0.03 (0.03–0.07)</td>
<td>0.22±0.03 (0.04–0.05)</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>1.58±0.18 (1.27–1.94)</td>
<td>2.15±0.18 (1.46–1.34)</td>
<td>1.38±0.18 (0.90–2.39)</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>3.04±0.27 (2.71–4.43)</td>
<td>2.17±0.27 (1.95–2.60)</td>
<td>4.63±0.27 (2.76–6.83)</td>
</tr>
<tr>
<td>LCP</td>
<td>5.16±0.51 (4.67–6.27)</td>
<td>5.01±0.51 (4.44–6.60)</td>
<td>18.6±0.51 (16.9–39.1)</td>
</tr>
<tr>
<td>(n-3)</td>
<td>5.41±0.49 (4.60–5.90)</td>
<td>5.26±0.49 (4.64–6.91)</td>
<td>6.35±0.49 (3.65–9.66)</td>
</tr>
<tr>
<td>(n-6)</td>
<td>7.89±0.78 (7.19–9.94)</td>
<td>10.3±0.78 (10.1–11.4)</td>
<td>9.91±0.78 (6.96–13.9)</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>2.17±0.15 (1.53–2.83)</td>
<td>1.89±0.15 (1.47–2.19)</td>
<td>1.62±0.15 (1.31–2.31)</td>
</tr>
<tr>
<td>18:4n-6</td>
<td>16.7±0.19 (11.9–15.5)</td>
<td>17.1±0.19 (15.8–17.3)</td>
<td>11.6±0.19 (8.63–13.8)</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>2.87±0.21 (1.84–3.85)</td>
<td>2.80±0.21 (2.24–3.16)</td>
<td>2.79±0.21 (1.93–4.00)</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>1.21±0.10 (0.73–1.82)</td>
<td>1.06±0.10 (0.63–1.11)</td>
<td>1.06±0.10 (0.65–1.57)</td>
</tr>
<tr>
<td>LCP</td>
<td>20.1±0.18 (18.1–23.4)</td>
<td>22.3±0.18 (20.3–23.3)</td>
<td>17.0±0.18 (13.9–20.5)</td>
</tr>
<tr>
<td>(n-6)</td>
<td>28.6±0.20 (27.2–31.0)</td>
<td>32.2±0.20 (30.2–34.3)</td>
<td>27.7±0.20 (26.1–31.1)</td>
</tr>
</tbody>
</table>

**Study groups.** The Maasai are a group of Nilotic pastoralists from the Maasai Steppe nearby the dispensary in Ruvu. Their diet is composed of curdled milk, some ugali (corn porridge), and meat. Because fish are considered as snake-like creatures, they are usually not eaten. The Pare group included Bantu women from the agricultural Pare and Sambaa tribes who visited same hospital from the nearby Pare Mountains. Their staple foods are ugali, rice, and corn–wheat pancakes, with some meat, fish, and beans, together with a relatively abundant consumption of fruits and vegetables. Fish consumption is irregular, because the nearest lake is located >30 km from their villages. The Sengerema group included Bantu women from the fishery families living on the southern shore of Lake Victoria who attended to the hospital of Sengerama. Ugali, cassava root, and plantain are staple foods, but consumption of fish is markedly more regular in the Lake Victoria area than for inhabitants of the Pare area. Smoking and alcohol consumption are very uncommon within these communities, especially among women. Importantly, included participants had neither the possibility to (Pare and Sengerena) nor major interest in (Maasai) deviation from their cultural habits, including their diets. We observed, and local doctors, nurses, and participating women confirmed, that neither pregnancy nor lactation was associated with any change in dietary habits in any of the investigated groups. The average dietary intakes of mothers at delivery as well as at 3 mo PP were therefore likely to be representative for the lifetime dietary habits of their respective ethnic group/trbe as a whole. Data on age, parity, fish consumption, and duration of lactation were obtained from the medical records or by interviews in Kiswahili. Gestational ages were checked by the last known menstrual period and fundal height. After delivery, all infants were routinely checked for signs of prematurity.

1 Values are means (range). At a time, medians in a row without a common letter differ. 2 Different from corresponding mother/infant; *different from delivery. All at P < 0.05.

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Groningen (The Netherlands) for FA analysis. FA were analyzed according to previously described methods (22). In short, analyses of FA-methyl esters were performed by capillary GC/flame ionization detection following the addition of an internal quantification standard (17:0), transmethylation, and extraction. FA were quantified on the basis of the added 17:0. FA compositions are expressed as g/100 g FA (g%) for consistency with current literature and the omega-3 index.

Statistics. Statistical analyses were performed with SPSS version 16.0.1. Between-group analyses were tested for normality and subsequently analyzed with an ANOVA and Student’s t test or a Kruskal-Wallis test and a Mann Whitney U test. Differences were considered significant at \( P < 0.05 \). Differences between mothers and infants were tested with a paired-samples t test or a Wilcoxon’s test. Differences were considered significant at \( P < 0.05 \). Bonferroni corrections were made for type-1 errors by dividing all \( P \)-values by the number of comparisons. Consequently, all differences with \( P < 0.05 \) divided by the number of comparisons were considered significant. Correlations were tested by linear regression analysis.

Results

Anthropometrics and fish intakes

We included 193 women/infant pairs in this study: 83 at delivery (63 complete pairs: 6 Maasai, 24 Pare, 33 Sengerema) and 110 after 3 mo of exclusive lactation (104 complete pairs: 8 Maasai, 36 Pare, 60 Sengerema). Drop-out was due to obtaining consent for only the mother or the infant, early discharge, and logistical and analytical imperfections. The anthropometrics and characteristics of the investigated mothers and their children are shown in Table 1. There were no between-group differences for the mothers at delivery or at 3 mo PP, except for a lower weight at delivery \( (P < 0.05) \) and higher length at all times \( (P < 0.05) \) of Maasai mothers compared with Pare mothers and a higher BMI of Sengerema mothers compared with Maasai mothers at 3 mo PP \( (P = 0.048) \). The principal relevant between-group maternal difference was in fish intakes: fish intakes of the Sengerema mothers at delivery and at 3 mo PP \( (4-5 \text{ times/wk}) \) were higher than of the Pare mothers \( (2–3 \text{ times/wk}; P < 0.03) \), which in turn were greater than of the Maasai mothers \( (0 \text{ times/wk}; P < 0.004) \).

Maternal and infant RBC-AA and DHA

Maternal vs. infant RBC-DHA and AA at delivery and at 3 mo PP. At delivery, RBC-DHA in Maasai mothers was not different compared with their infants but became lower \( (P = 0.022) \) at 3 mo PP. Pare mothers tended to have lower RBC-DHA at delivery \( (P = 0.091) \) and had lower RBC-DHA at 3 mo PP compared with their infants \( (P < 0.001) \). Compared with their infants, Sengerema mothers had higher RBC-DHA at delivery \( (P = 0.029) \) but lower DHA at 3 mo PP \( (P = 0.001) \) (Table 2, Fig. 1A). Maasai maternal RBC-AA was lower at delivery compared with their infants \( (P = 0.028) \) but higher at 3 mo PP \( (P = 0.015) \). RBC-AA of Pare mothers was lower at delivery \( (P < 0.001) \) compared with their infants, but there was no difference at 3 mo PP \( (P = 0.12) \). RBC-AA of the Sengerema mothers was lower than that of their infants at delivery \( (P < 0.001) \) and at 3 mo PP \( (P < 0.001) \) (Table 2, Fig. 1B).

Delivery vs. 3 mo PP RBC-DHA and AA in mothers and infants. Both Maasai mothers \( (P = 0.003) \) and their infants \( (P = 0.039) \) had higher RBC-DHA at delivery compared with 3 mo PP. From delivery to 3 mo PP, Pare mothers had a decrease in RBC-DHA \( (P < 0.001) \), but infant RBC-DHA did not decrease during lactation \( (P = 0.16) \). RBC-DHA in Sengerema mothers decreased after delivery \( (P = 0.001) \) but increased in their infants \( (P = 0.027) \) (Table 2, Fig. 1A).

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both Maasai mothers \((P < 0.001)\) and their infants \((P = 0.004)\) had lower RBC-DHA compared with Pare mothers and infants. Sengerema mothers and their infants had higher RBC-DHA compared with their Pare counterparts at delivery and at 3 mo PP \((P < 0.001)\). RBC-DHA of Sengerema mothers and their infants were also higher compared with their Maasai counterparts at all times \((P < 0.001)\) (Table 2, Fig. 1A).

Maternal RBC-AA was higher in Maasai compared with Pare mothers at delivery \((P = 0.005)\) and at 3 mo PP \((P < 0.001)\), but RBC-AA was not significantly different between Maasai and Pare infants at delivery or at 3 mo PP. RBC-AA in Sengerema mothers was higher \((P = 0.049)\) compared with Pare mothers at delivery, but there was no difference at 3 mo PP. Also, there were no differences in infant RBC-AA between Sengerema and Pare at delivery or at 3 mo PP. RBC-AA was lower in Sengerema compared with Maasai mothers at delivery \((P = 0.042)\) and at 3 mo PP \((P < 0.001)\). Sengerema and Maasai infants did not differ in RBC-AA at delivery or at 3 mo PP (Table 2, Fig. 1B).

**Maternal vs. infant RBC-DHA and maternal vs. infant RBC-AA**

When the 3 groups were combined, there were significant relations between maternal and infant RBC-DHA at delivery (Fig. 2A) and at 3 mo PP (Fig. 2C). Data that were previously reported for mother-infant pairs in Dominica (23) and The Netherlands (24) fitted well within the relationships (Fig. 2A, B). Maternal RBC-DHA explained 61% of the variation in infant RBC-DHA at delivery and 76% at 3 mo PP. In contrast, there were no significant relations between maternal RBC-AA and infant RBC-AA at delivery (Fig. 2B) or at 3 mo PP (Fig. 2D).

Using the equations shown in Figure 2, at \(y = x\), we calculated that at delivery, maternal RBC-DHA levels < 5.6 g% corresponded with higher infant RBC-DHA, whereas infant RBC-DHA was lower than maternal RBC-DHA at ≥5.6 g% (Fig. 2A). At 3 mo PP, maternal RBC-DHA levels < 7.9 g% corresponded with higher infant RBC-DHA. There were few mothers with RBC-DHA > 7.9 g%, but extrapolation suggests that the reverse may occur beyond this maternal RBC-DHA status (Fig. 2C).

**Changing PP maternal RBC-DHA as a function of RBC-DHA at delivery.** The fractional changes in maternal RBC-DHA from delivery to 3 mo PP as a function of maternal RBC-DHA at delivery (Fig. 4A) and infant RBC-DHA at delivery (Fig. 4B) were derived from the combined Maasai, Pare, and Sengerema participants. Fractional changes are given as the ratio maternal

**Maternal and infant RBC-DHA vs. RBC-AA**

The relations between RBC-DHA and RBC-AA for the mothers and the infants at delivery and at 3 mo PP are presented in Figure 3. We first analyzed the DHA compared with AA relations in each of the 3 tribes. Linear regression analyses (Table 3; Fig. 3) showed different slopes for the Maasai, Pare, and Sengerema. Those of the Maasai and Pare were mostly positive, whereas those in Sengerema were mostly negative. Pooling of all data suggested that the relations between RBC-DHA and RBC-AA were at best bell shaped and were therefore fitted in a quadratic function (Table 3; Fig. 3). The calculated curves reached their summits at 5.7, 5.7, 4.8, and 6.2 g% RBC-DHA (Fig. 3A–D, respectively). The best linear fit from a RBC-DHA above the respective summits is also in Table 3. Taken together, the relations were positive up to ~6 g% RBC-DHA and became negative or nonsignificantly positive beyond ~6 g%.
RBC-DHA at 3 mo PP: maternal RBC-DHA at delivery, in which a ratio < 1 indicates that the mother is in negative DHA balance during lactation and > 1 indicates that the mother is in positive postnatal DHA balance. By extrapolation, we found that a maternal RBC-DHA equilibrium from delivery to 3 mo PP was reached when the maternal RBC-DHA was 8.1 g% at delivery (Fig. 4A). This maternal RBC-DHA equilibrium corresponded with an infant RBC-DHA of 7.1 g% to 7.2 g% at delivery and 8.0 g% at 3 mo PP (calculated from Fig. 2A). Using the 7.2 g% from Figure 4B in Figure 2A, we calculated that maternal postnatal RBC-DHA equilibrium would occur when the mother reaches an RBC-DHA of 8.3 g% at delivery, which is close to the 8.1 g% from Figure 4A. Taken together, a PP DHA equilibrium in fully lactating mothers was reached at a maternal RBC-DHA of 8 g% at delivery, which corresponded with a maternal RBC-DHA of 8 g% at 3 mo PP and an infant RBC-DHA of 7 g% at delivery that increased to 8 g% at 3 mo PP.

**Discussion**

In this study of 3 populations of mother-infant pairs with different maternal consumption of freshwater fish (Table 1, Fig. 1A), we found that biomagnification occurs up to 8 g% DHA in maternal RBC and maternal RBC-DHA equilibrium is reached at 8 g%. This maternal RBC-DHA equilibrium corresponds with an infant RBC-DHA of 7 g% at delivery that increases to 8 g% during 3 mo of exclusive lactation. Unlike RBC-DHA, maternal and infant RBC-AA are independently regulated in these populations, and bell-shaped RBC-DHA vs. RBC-AA relations might support uniform infant RBC-AA.

**DHA.** Although maternal RBC-DHA decreased during breastfeeding in all groups, the decline was more pronounced in the Maasai and Pare, who have lower fish intakes. The PP change in infant RBC-DHA was dependent on the maternal RBC-DHA status and decreased (Maasai), did not change (Pare), or increased (Sengerema) (Fig. 3). The decrease in infant RBC-DHA in the Maasai population was significant when compared to the other two groups (Table 3). The increase in infant RBC-DHA in the Pare population was significant when compared to the Maasai population (Table 3).

**TABLE 3** Maternal RBC-DHA and infant RBC-DHA vs. RBC-AA at delivery and at 3 mo PP

<table>
<thead>
<tr>
<th>Figure</th>
<th>Maasai linear</th>
<th>P1</th>
<th>Pare linear</th>
<th>P</th>
<th>Sengerema linear</th>
<th>P</th>
<th>Combined data quadratic</th>
<th>P</th>
<th>Summit of quadratic fit2</th>
<th>Combined data &gt; summit (linear)4</th>
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<td>0.16x + 13.6</td>
<td>NS</td>
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<td>0.04</td>
<td>−0.43x + 15.6</td>
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<td>8.49 + 1.47x−0.013x2</td>
<td>0.07</td>
<td>5.7</td>
<td>−0.61x + 16.9</td>
<td>0.003</td>
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<tr>
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<td>0.05x + 16.2</td>
<td>NS</td>
<td>0.59x + 13.1</td>
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<td>−0.52x + 19.6</td>
<td>0.025</td>
<td>9.62 + 2.41x−0.21x2</td>
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<td>11.1 + 1.33x−0.14x2</td>
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<tr>
<td>3D</td>
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<td>0.72x + 11.3</td>
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<td>0.001</td>
<td>6.2</td>
<td>0.15x + 13.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

1 The correlation RBC-DHA vs. RBC-AA with the highest R² is shown for each separate group (linear relations); for the combined data (quadratic relations) and for all data with RBC-DHA > summit of the quadratic function (linear relations).
2 Significance of the relationship, differences were considered significant if P < 0.05; borderline significance is also shown (0.1 ≥ P ≥ 0.05); NS, P > 0.1.
3 Calculated maximum/summit of the quadratic relation.
4 Equation of the linear function applied to all data beyond the calculated maximum of the quadratic relation.
when maternal RBC-DHA is biomagnification. Postnatal maternal DHA equilibrium is reached at an infant RBC-DHA of 6 g% at delivery to 8 g% at 3 mo PP, respectively; a ratio of maternal RBC-DHA at 3 mo PP:

maternal RBC-DHA at delivery. Data are means ± SEM, n = 34 and 60 for Pare infants at delivery and 3 mo PP, respectively; n = 36 and 61 for Sengerema infants at delivery and 3 mo PP, respectively. A ratio of maternal RBC-DHA at 3 mo PP: maternal RBC-DHA at delivery < 1 indicates that the mother is in negative DHA balance during lactation; a ratio > 1 indicates that the mother is in positive postnatal DHA balance.

The peculiar mother-infant sharing of DHA during pregnancy and lactation is presented in simplified form in Figure 5. Postnatal infant DHA equilibrium occurs at an infant RBC-DHA of ~6 g% at delivery (Fig. 5A), which corresponds with a maternal RBC-DHA of ~6 g% at delivery and 6 g% at early pregnancy (M. Luxwolda, R. Kuipers, W. Sango, G. Kwesigabo, D. Dijck-Brouwer, F. Muskiet, unpublished data). Postnatal infant DHA equilibrium coincides with a mature milk DHA level of ~0.4 g% (R. Kuipers, unpublished data). Under these circumstances, the mother loses DHA during lactation to reach a maternal RBC-DHA of ~5 g% after 3 mo. This can be calculated by entering an infant RBC-DHA of 6 g% into Figure 3C or 4B and a maternal RBC-DHA of 6 g% into Figure 4A. The combination of infant DHA equilibrium and maternal DHA losses during lactation can be considered as postnatal DHA biomagnification. Postnatal maternal DHA equilibrium is reached when maternal RBC-DHA is ~8 g% at delivery (Figs. 3A, 5B), which corresponds with 8 g% at early pregnancy (M. Luxwolda, R. Kuipers, W. Sango, G. Kwesigabo, D. Dijck-Brouwer, F. Muskiet, unpublished data) and a mature milk content of ~1 g% DHA (R. Kuipers, unpublished data). Under these circumstances, infant RBC-DHA is ~7 g% at delivery (Fig. 3B), which we named bioattenuation (M. Luxwolda, R. Kuipers, W. Sango, G. Kwesigabo, D. Dijck-Brouwer, F. Muskiet, unpublished data). This intrauterine bioattenuation occurs despite the rapid postnatal infant RBC-DHA increase to adult levels of 8 g% during the first 3 mo of lactation (Fig. 2C) and illustrates the apparent importance of limiting DHA transplacental transport at high fish intakes, which is followed by a postnatal mother-to-child DHA surge via the milk.

Although there is consensus on recommending an average DHA intake of at least 200 mg/d during pregnancy and lactation (16), the optimal DHA status for the mother and her infant is currently unknown. Intakes of fish and fish oils during pregnancy result in slightly longer gestation, marginally higher birth weight, and a reduced risk of preterm delivery (16). However, RCT with DHA during pregnancy targeting infant neurodevelopment are less clear. The largest RCT so far with cod liver oil (1200 mg DHA/d) supplemented to a baseline diet containing 200–300 mg DHA/d (25–27) showed no differences in cognitive development at 6 and 9 mo and a promising higher IQ at 4 y of age but not at 7 y. These outcomes were in line with the negative outcomes for associations between umbilical plasma PL- and RBC-DHA and infant cognitive development at 4 and 7 y (28,29). Supplementation with fish oil or DHA in pregnancy, however, might benefit infant visual maturation and acuity (30–32) and newborn sleep pattern maturity (33). Thus, the inconsistent results of maternal DHA supplementation studies contrast with positive associations between neonatal brain DHA and cognitive and behavioral performance noted in

![Figure 4](https://academic.oup.com/jn/article-abstract/141/3/418/4743589/424)

**FIGURE 4** Maternal RBC-DHA at delivery (A) and infant RBC-DHA at delivery (B) compared with the ratio of maternal RBC-DHA at 3 mo PP/maternal RBC-DHA at delivery. Data are means ± SEM, n = 6 and 9 for Maasai mothers; n = 8 and 8 for Maasai infants at delivery and 3 mo PP, respectively, n = 27 and 38 for Pare mothers, 29 and 38 for Pare infants at delivery and 3 mo PP, respectively; n = 34 and 60 for Pare mothers, 36 and 61 for Sengerema mothers, 36 and 61 for Sengerema infants at delivery and 3 mo PP, respectively. A ratio of maternal RBC-DHA at 3 mo PP: maternal RBC-DHA at delivery < 1 indicates that the mother is in negative DHA balance during lactation; a ratio > 1 indicates that the mother is in positive postnatal DHA balance.

![Figure 5](https://academic.oup.com/jn/article-abstract/141/3/418/4743589/424)

**FIGURE 5** Synoptic overview of the infant (A) and maternal (B) RBC-DHA equilibrium during 3 mo lactation. (A) Infant PP RBC-DHA equilibrium occurs at 6 g% DHA and coincides with depletion of maternal DHA from 6 g% at delivery to 5 g% DHA at 3 mo PP; a maternal RBC-DHA of 6 g% at 3 mo PP coincided with 0.4 g% DHA in milk. (B) Maternal PP RBC-DHA equilibrium occurs at 8 g% DHA, which coincides with an increase in infant RBC-DHA from 7 g% DHA at delivery to 8 g% at 3 mo PP; a maternal RBC-DHA of 8 g% coincided with 1.0 g% DHA in milk.
combined human and animal studies (2). Discrepancies might relate to short-term supplementation of relatively low doses of DHA, differences in frequencies of polymorphisms in the desaturase enzymes, and lack of dose adjustment to differences in individual baseline maternal and infant DHA status.

Epidemiological studies have linked fish consumption, EPA+DHA intakes, and EPA+DHA status to a reduction in affective disorders, cognitive impairment, Alzheimer’s disease, and PP depression (7). An RBC-(EPA+DHA) ≥8 g%, as found in healthy Japanese participants, seems an appropriate target to minimize major depressive disorders and bipolar depression (34), but the relation between (n-3) LCP and PP depression has not been substantiated by RCT (18,19). With regard to cardiovascular disease, an RBC-DHA > 8 g% (omega-3 index > 10 g%) was associated with the lowest risk for acute coronary syndrome and sudden cardiac death (35–37). (n-3) LCP intakes > 450 mg/d were shown to be beneficial for lowering heart rate, blood pressure, and TG and to reach maximum antithrombotic effects (38). During 2.5 million years of evolution, our genome has become adapted to a diet high in both (n-3) and (n-6) LCP (8). The presumed high LCP intakes by our ancestors likely resulted in development of maternal stores of sufficient magnitude to prevent depletion during lactation and to sustain DHA transfer to the developing infant (39). The high DHA in milk and RBC that were observed in populations with high fish consumption are consistent with the high intakes of AA, EPA, and DHA from our Paleolithic diets (8).

It has been suggested that the RBC membrane might reach saturation at levels between 8 and 10 g% DHA (40,41). Supplementation of lactating women with doses up to 1.3 g DHA/d dose-dependently increased maternal RBC-DHA to 7.9 g%, milk DHA to 1.13 g%, and infant RBC to 9.1 g% DHA. Whereas milk DHA continued to increase, no further increase in infant RBC-DHA from ~0.8 g% DHA in milk was seen, corresponding to a maternal-infant RBC-DHA of 8–10 g% (40,41). However, we have little data (Fig. 2A,C) to show the relation between infant and maternal RBC-DHA beyond 8 g% DHA.

**AA.** Delivering and lactating women with low, intermediate, and high fish intakes proved to have different RBC- AA status at both delivery and after 3 mo (Fig. 1B), although RBC- AA increased in all maternal groups after delivery. In contrast, RBC- AA was remarkably similar for all infant groups at delivery and after 3 mo and decreased consistently after delivery (Fig. 1B). Biomagnification of AA across the placenta is clearly shown in Fig. 2B. However, the higher infant RBC-AA, compared with maternal RBC-AA, vanished within 3 mo of lactation (Fig. 2D). The concomitant increase in maternal RBC- AA might derive from discontinued utilization of AA by the placenta (42), discontinued transport to the fetus, or both. The PP decrease in infant RBC- AA might be consistent with the PP changes in the infant’s RBC-PL species (43). Mechanistically, it might result from the discontinued AA transport across the placenta and from the change of hormonal milieu that accompanies delivery, which is likely to influence AA enzymatic activities. It was shown that the infant’s LCP synthetic activity decreases drastically after delivery (44). Even high milk AA contents, such as in the Pare (0.80 g%; R. Kuipers, unpublished data), did not prevent a decrease in infant RBC-AA. This raises the question whether milk AA is at all intended to sustain infant AA status after delivery. The remarkable between-group similarity of infant RBC-AA levels at delivery and to a lesser extent at 3 mo PP rather suggests a well-controlled fetal AA status that gradually assumes adult levels after delivery. This suggestion is in line with Hsieh et al. (39), who recently showed that CNS AA levels in baboon neonates are tightly controlled at the level of incorporation or utilization, that CNS AA levels were unaffected by dietary AA and that AA decreased in all CNS structures with age.

**DHA vs. AA.** We recently suggested a synergistic relation between DHA and AA at low DHA status and an antagonistic relationship between DHA and AA at high DHA status (45). Such a relationship was previously proposed by Horrobin et al. (3). Our data on the antagonistic relation between DHA and AA were, among others, based on their contents in RBC. These suggested that RBC-DHA increasingly suppresses RBC-AA from an RBC-DHA level of ~6 g% (45). Interestingly, in the present study, we also found synergy between DHA and AA below an RBC-DHA of ~6 g% and antagonism beyond 6 g% (Fig. 3). This suggests that in Maasai and Pare infants born to mothers with low fish intakes, biomagnification of DHA across the placenta indirectly caused a synergistic increase of RBC-AA by virtue of higher transplacental DHA transport, whereas in the Sengerema infants born to mothers with high fish intakes, bioattenuation of DHA across the placenta indirectly caused a diminished antagonistic decrease of RBC-AA. In other words, both biomagnification and bioattenuation during pregnancy may aim at a certain fetal AA status. The subsequent postnatal DHA surge via the milk may be regarded as a form of postnatal biomagnification. This DHA surge was apparently unable to prevent a postnatal RBC-DHA decrease in Maasai infants, caused a nonsignificant RBC-DHA decrease in Pare infants, and an increase in Sengerema infants. Analogous to the intrauterine period, the resulting low DHA status in Maasai infants might have synergistically lowered their RBC-AA, whereas the high DHA status of the Sengerema infants might have lowered their RBC-AA in an antagonistic manner. The presumed synergy and antagonism may in this manner have contributed to the observed low inter-individual variation of RBC-AA at 3 mo PP. It is possible that their existence illustrates the important role of AA for the developing infant during pregnancy and the increasing postnatal importance of DHA in the suppression of AA.

It might be considered a limitation that we interpreted the differences between the subgroups at delivery and 3 mo in terms of longitudinal changes. This assumption might, however, be justified by documented lifetime stable dietary intakes in these populations, while in essence no different results have been noted with literature data derived from genuine prospective studies. Our interpretations rest on the reliability of RBC-LCP status as a proxy for whole body LCP status. The RBC-FA composition is, however, widely considered a reliable reflection of whole body status (46), particularly in populations with stable lifetime dietary habits. Maternal to infant FA transport may be influenced by placental size; however, we observed no between-group differences for placental size or architecture while assisting deliveries. Finally, we interpreted static values in terms of fluxes. Any of these interpretations may consequently be biased and should be confirmed by data from tracer or other dynamic studies.

We conclude that the postnatal DHA surge via the milk represents a genuine form of postnatal biomagnification that occurs up to a maternal RBC-DHA status of 8 g%. This contrasts with biomagnification via the placenta, which occurs up to a maternal RBC-DHA status of 6 g%. This discrepancy, together with the postnatal increase in maternal RBC-AA and concurrent decrease in infant RBC-AA, might indicate a switch...
of the importance of AA during gestation to DHA during lactation. Unlimited DHA transfer at high maternal DHA status might be undesirable because of its antagonistic effect on AA, while the PP DHA surge via the milk might at the suppression of AA by DHA. An RBC-DHA of 8 g% in adults and the rapid PP increase of infant RBC-DHA to 8 g% might support infant neurological development and prevent adult diseases linked to low fish intakes. Low DHA status might be associated with diseases in the peripartum period, but this needs confirmation from RCT targeting 8 g% RBC-DHA.

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Literature Cited


