Different fatty acid composition of serum phospholipids of small and appropriate for gestational age preterm infants and of milk from their mothers

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Abstract

Background: Placental supply of fatty acids (FA) is essential for normal foetal development but in premature infants this supply is interrupted. To investigate the association of intrauterine growth restriction with serum phospholipid and breast milk FA composition, we compared preterm infants small for gestational age (SGA) and matched appropriate for gestational age (AGA), and their mothers’ milk during the first 4 weeks of postnatal life.

Methods: Sera from 11 SGA and 12 AGA infants born 34-36 weeks of gestation were collected at birth, 14th and 28th day, and breast milk on 14th and 28th day after birth. FA composition was analyzed by gas chromatography.

Results: Preterm SGA infants had significantly lower oleic, total monounsaturated FA (MUFA), docosahexaenoic acid (DHA) and n-3 polyunsaturated FA (PUFA) and higher levels of stearic and linoleic acid at birth than AGA infants (p<0.05). DHA was significantly lower, whereas docosatetraenoic and docosapentae noic acids were higher in SGA infants after 28 days. Mothers of AGA infants had markedly lower levels of MUFA and higher levels of total and n-6 PUFA in their breast milk.

Conclusion: SGA infants have altered serum phospholipid FA composition at birth and during their first month of life, probably due to inadequate transplacental supply and activity of desaturase system. Results on human milk suggest that pregnancies with AGA or SGA would later influence breast milk FA composition. Hippokratia 2012; 16 (3): 230-235

Key words: preterm infants, small for gestational age, breast milk, phospholipids, fatty acids

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Introduction

Polyunsaturated fatty acids (PUFA) are vitally important structural elements of cell membranes. Apart from membrane lipid structure and function, both n-6 and n-3 series PUFA are associated with many other important functions including intracellular signalling, plasma lipid transport, covalent modification of cellular proteins, gene expression (transcription, mRNA stability) and cellular differentiation1. They are also substrates for cyclooxygenase and lipoxygenases and precursors for a variety of eicosanoids1. Additionally, PUFA are essential in the formation of new tissues during pregnancy and foetal development2.

Fatty acids (FA) in placenta originate primarily from maternal triglycerides hydrolysed by lipoprotein lipase and from nonesterified FA in the maternal circulation3. The pattern of PUFA transferred from the mother to the foetus depends on maternal stores, maternal dietary intakes, and metabolic processes in the placenta4. During the last trimester of pregnancy, a substantial number of essential FA, predominantly the long-chain PUFA (LCP-UFA) are transferred from the mother to the foetus5,6. At the same time, foetus is also getting capable to directly synthesize LCPUFA from their precursors, but the quantitative degree of synthesis is not clear7. Therefore, the placental supply of LCPUFA is critical for the synthesis of structural lipids, and to normal foetal development8.

After birth, the placental supply is interrupted. In premature infants, who are not capable to synthesize LCPUFA sufficiently, concentration of LCPUFA rapidly decreases; thus prematurity directly reduces the LCPUFA bioavailability7. A significantly lower LCPUFA status has been observed in pre-term infants (born between 26th-36th week of gestation) appropriate for gestational age (AGA) than in full term infants3, but also in term infants with intrauterine growth restriction (IUGR) than in AGA infants6. As a result of IUGR due to reduction or an arrest of growth processes at various stages during the foetal life, infants at birth are small for gestational age (SGA), which is below the 10th percentile of expected weight, compared with infant counterparts with birth weight appropriate for gestational age.
In addition to differences in FA profile at birth, postnatal deficit of LCPUFA in infants may be a result of an inadequate dietary supply or of an impaired endogenous synthesis from dietary precursors. Postnatal deficits in n-3 PUFA have been associated with neural and retinal complications, while postnatal deficits in n-6 PUFA have been related to infant growth, liver and kidney function, heart contractility, and skin permeability. In contrast to standard infant formulas and cow’s milk, human milk contains all essential FA and is the best source of LCPUFA for infants.

Regarding the importance of FA for normal foetal development, the aims of our study were:
1) to determine and compare the profiles of serum phospholipid FA during first 4 weeks after birth in AGA and SGA infants;
2) to determine and compare FA composition of breast-milk during the first 4 weeks of lactation in mothers of AGA and SGA infants.

Materials and Methods

Subjects

Twenty-three preterm infants born between 34th and 36th week of gestation with birth weight from 1650g to 2200g were recruited from the Neonatal Care Unit of the Belgrade Institute for Neonatology (Serbia). Entry criteria were: healthy and clinically stable (Apgar at 5 min >7) and parental informed approval of infant participation obtained after birth. Exclusion criteria were maternal diseases, gestational diabetes, preeclampsia, alcohol or drug abuse, abnormal foetal karyotype, and foetal malformations or infections. All pregnancies were singleton and none of the women smoked during pregnancy, to avoid potential influence of smoking on FA profiles in both mothers and infants. All babies were exclusively breast fed by their mothers.

Maternal characteristics (Table 1) were drawn from obstetrical charts. Gestational age (GA) was determined using three parameters: the last menstrual period of the mother, ultrasound during an early stage of pregnancy and a clinical evaluation at birth according to the modified Ballard evaluation.

Infants were weighed naked within 30 min after delivery and before the first feeding, by an electronic integrating scale (Sartorius, AG, Gottingen, Germany; precision ±5.0 g). Crown-to-heel length was measured on a recumbent infant board to the nearest millimetre by a trained operator using a Harpenden (UK) neonatometer. Cranial circumference was measured with a flexible narrow steel tape which was applied firmly around the head above the supraorbital ridges. Infants were classified as AGA (12 infants) and SGA (11 infants) respectively, according to Lubchenco standard.

All mothers provided written informed consent which was approved by the Ethical Review Boards of the participating institutions in accordance with the principles of the Declaration of Helsinki.

Fatty acid analysis

Fatty acids in serum phospholipids (PL) and human milk were analyzed by gas chromatography. Blood samples (0.4ml) were taken from the peripheral vein 1.5 - 2 hours after feeding on the 1st, 14th and 28th day of postnatal life. Serum was separated by centrifugation and the FA profile was analyzed immediately.

In the morning and after feeding the infants, breast-milk (5-10ml) was manually collected into a sterile vessel on the 14th and the 28th day of lactation. FA were analyzed immediately.

The total serum lipids were extracted from 200 μl of the infant’s serum using the Folch method. The PL fraction was isolated from the extracted serum lipids by one-dimensional thin-layer chromatography. Methyl esters of serum phospholipid FA were prepared using a method which has been described and published. The fatty acid methyl ester derivatives were then analyzed by gas chromatography (GC) using a Varian GC (model 3400) as described. The individual fatty acid methyl esters in our samples were identified using the retention times of authentic standards (Sigma Aldrich Chemie, Taufkirchen, Germany) and/or polysaturated fatty acid (PUFA-2) standard mixture (Supelco, Inc. Belleforte, USA).

Milk FA were extracted from 500 μl of human milk using the 4.5 ml chloroform-methanol mixture (2:1 v/v). One mL of lipid extract was methylated and then analyzed for total FA content in milk using GC. Fatty acid methyl esters were identified the same way as for the serum PL.

Statistical analysis

All results are expressed as the mean ± SD. Normality was tested using the Shapiro-Wilks test. Maternal characteristics were compared by the Student t-test. FA profiles comparison between AGA and SGA groups was made using
the unpaired Student’s t-test for normally distributed variables and Mann Whitney U test for non-normally distributed variables. One way ANOVA, followed by the Tukey post hoc test was used for the comparisons among FA profiles at birth, at 14th and 28th day of postnatal life. Differences were considered significant at p-values of ≤0.05.

Results
A total of 23 preterm infants, all breastfed, entered the study. According to birth-weight they have been divided into 11 mildly preterm SGA and 12 mildly preterm AGA. Maternal and anthropometric characteristics of infants are listed in Tables 1 and 2 respectively. Mothers of SGA infants were older than those of AGA infants, while all other anthropometric characteristics were similar in the two groups. The weight and length were higher in AGA infants at birth, as well as 14th and 28th days of postnatal life. Differences were considered significant at p-values of ≤0.05.

Results

Postnatal changes in FA profile of serum phospholipids during first 4 weeks of postnatal life in AGA and SGA infants
Our results showed a significant decrease of palmitic (16:0), palmitoleic (16:1), saturated FA (SFA) and MUFA followed by a significant increase in linoleic acid, n-6 and total PUFA after 2 and 4 weeks of postnatal life in both groups of infants, when compared to the levels at birth. However, content of n-3 PUFA and particularly DHA were significantly lower after 2 and 4 weeks in SGA infants (Table 3).

Fatty acid composition in breast milk at 14th and 28th day of lactation
FA composition of human milk is presented in Table 4. As it can be seen from Table 4, breast milk of mothers of preterm AGA infants is significantly different from milk of mothers of SGA infants at 14th as well as 28th days of lactation. The n-6/n-3 ratio was significantly higher in mothers of AGA than SGA infants after 28 days of lactation.

Changes in FA composition of human milk during the first 4 weeks of lactation
During the first 4 weeks of lactation, milk of all mothers changed but not in the same way in both groups of mothers. In mothers of SGA infants, levels of almost all saturated FA in breast milk significantly increased, while oleic acid, DHA and DTA (22:4n-6) decreased between 2 and 4 weeks of lactation. In milk of mothers with AGA preterm infants proportion of stearic acid significantly increased and palmitoleic acid, 20:3n-6 and 17:0 significantly decreased (Table 4).

Discussion
Our study followed serum phospholipid FA profiles in AGA and SGA preterm infants at birth and during their first 4 weeks of life, as well as FA composition of their mothers’ breast milk at the same time points. To our

Table 2: The anthropometric measures of infants during first 28 days of life

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Length (cm)</th>
<th>Head circumference (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>at birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA group</td>
<td>2125.00±417.33</td>
<td>43.62±3.20</td>
<td>30.87±1.44</td>
</tr>
<tr>
<td>SGA group</td>
<td>1806.67±20.82*</td>
<td>41.33±0.58*</td>
<td>31.00±1.00</td>
</tr>
<tr>
<td><strong>14th day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA group</td>
<td>2112.50±471.48</td>
<td>44.53±2.40</td>
<td>31.44±0.88</td>
</tr>
<tr>
<td>SGA group</td>
<td>1876.67±107.86**</td>
<td>42.05±0.67***</td>
<td>31.00±0.50</td>
</tr>
<tr>
<td><strong>28th day</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGA group</td>
<td>2650.00±313.25</td>
<td>48.00±2.00</td>
<td>33.67±0.58</td>
</tr>
<tr>
<td>SGA group</td>
<td>2158.33±72.17***</td>
<td>44.00±1.00***</td>
<td>34.00±0.50</td>
</tr>
</tbody>
</table>

AGA, appropriate for gestational age; SGA, small for gestational age. Values are expressed as mean ± SD. Significant difference between AGA and SGA groups, *p<0.05, **p<0.01, ***p<0.001.
knowledge, this is the first study on serum phospholipid FA status in preterm infants at birth subdivided into SGA and AGA groups. In addition, there is a lack of literature data on breast milk fatty acid composition in mothers of preterm SGA infants.

FA levels in plasma PL at birth reflects foetus prenatal supply with FA\(^{10}\) thus it depends on mothers plasma FA profile and placental transport\(^{25}\). As mammals are unable to synthesize LA and \(\alpha\)-linolenic acid (ALA, 18:3\(n\)-3), which are precursors of the n-6 and n-3 families, the supply to the fetus and newborn of this FA depends on the dietary intake and the mother’s storage\(^{21}\).

The analysis of the FA composition in our study showed that LA was higher, whereas the major metabolic products of n-6 series arachidonic acid was lower in preterm SGA when compared with the AGA group at birth, indicating that intrauterine growth may affect the serum phospholipid PUFA levels. Contrary results were reported by Agostoni et al\(^{22}\), who found slightly higher LA and ALA but lower their major metabolic products arachidonic acid and DHA than AGA infants, suggesting that optimal biosynthetic performances and/or complete metabolic transfers from the maternal compartment to infants may take place only in case of term delivery and adequate intrauterine growth\(^{23}\). Other studies also reported lower essential FA status in IUGR babies, most of them premature, than in AGA group but measured in the umbilical cord blood, artery-vein wall, and placenta\(^{21,24}\). Lower levels of total MUFA in whole blood in term SGA infants compared to AGA infants were also reported, speculating that SGA infants near the term could increase the relative use of monoenes for energy production, considering the reduced fat stores in intrauterine growth retardation coupled with a less efficient energy production from glucose\(^{25}\). The biologic relevance of this observation is still unclear. In accordance to their findings, we found lower percentage of oleic acid and tolinolenic acid in the AGA group but the ratio n-6/n-3 PUFA in AGA group was lower than in SGA group.

During postnatal period, PUFA status of breastfed infants depends on milk FA composition, which varies considerably in different countries related to the mothers’ diet\(^{26}\). In industrialized countries, the consumption of n-6 FA has substantially increased during the last 50 years, whereas it has declined for the n-3 series\(^{27}\). Thus, in well-developed European countries, the ratio of n-6/n-3 PUFA in colostrum varies between 5 and 15\(^{28}\). Our results demonstrated an even higher n-6/n-3 PUFA ratio in mature milk of >20, especially in AGA mothers, indicating a higher dietary intake of n-6 linoleic acid and lower dietary intake of n-3 FA and marine product in Serbian population. The similar situation could be expected in the whole

<table>
<thead>
<tr>
<th>Fatty acids %</th>
<th>AGA group at birth</th>
<th>SGA group at birth</th>
<th>AGA group 14th day</th>
<th>SGA group 14th day</th>
<th>AGA group 28th day</th>
<th>SGA group 28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>34.1±3.68</td>
<td>32.7±3.43</td>
<td>28.6±2.50(^{bc})</td>
<td>28.0±0.73(^{bc})</td>
<td>26.4±1.85(^{ccc})</td>
<td>26.0±1.86(^{ccc})</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>1.58±0.24</td>
<td>1.12±0.29</td>
<td>0.52±0.17(^{bc})</td>
<td>0.6±0.15(^{bc})</td>
<td>0.40±0.12(^{ccc})</td>
<td>0.36±0.26(^{ccc})</td>
</tr>
<tr>
<td>18:0</td>
<td>36.2±0.91</td>
<td>16.2±0.61(^{***})</td>
<td>16.3±0.76(^{b})</td>
<td>16.8±0.79</td>
<td>16.7±1.33(^{ac})</td>
<td>18.5±0.63(^{ac})</td>
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<tr>
<td>18:1n-9</td>
<td>11.9±0.73</td>
<td>10.4±0.98(^{a})</td>
<td>9.3±0.79</td>
<td>9.7±1.08(^{b})</td>
<td>10.5±2.15</td>
<td></td>
</tr>
<tr>
<td>18:1n-7</td>
<td>3.6±0.42</td>
<td>3.07±0.25</td>
<td>2.50±0.39(^{ab})</td>
<td>2.39±0.32</td>
<td>2.22±0.21(^{bc})</td>
<td>1.75±0.34(^{bc})</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>9.1±0.128</td>
<td>10.14±3.34(^{a})</td>
<td>22.8±2.46(^{bb})</td>
<td>24.0±2.75(^{bb})</td>
<td>24.46±3.69</td>
<td>24.49±1.61(^{bc})</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>3.16±0.35</td>
<td>3.45±0.51</td>
<td>2.86±0.54</td>
<td>2.98±0.68</td>
<td>3.12±0.78</td>
<td>2.91±0.40</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>19.2±3.85</td>
<td>18.00±2.42</td>
<td>12.91±1.44</td>
<td>11.96±2.19(^{bb})</td>
<td>12.64±2.15(^{bc})</td>
<td>12.22±1.04(^{bc})</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.17±0.08</td>
<td>0.18±0.15</td>
<td>0.12±0.06</td>
<td>0.2±0.07</td>
<td>0.19±0.13</td>
<td>0.21±0.16</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>0.83±0.34</td>
<td>0.76±0.10</td>
<td>0.55±0.14</td>
<td>0.66±0.12</td>
<td>0.64±0.17</td>
<td>0.95±0.29(^{a})</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.28±0.14</td>
<td>0.30±0.16</td>
<td>0.31±0.09(^{b})</td>
<td>0.34±0.02</td>
<td>0.41±0.07(^{cc})</td>
<td>0.52±0.10(^{ac})</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>3.72±1.02</td>
<td>2.86±0.61(^{a})</td>
<td>2.60±0.59</td>
<td>2.13±0.37(^{a})</td>
<td>2.86±0.55</td>
<td>2.17±0.49(^{ac})</td>
</tr>
<tr>
<td>SFA</td>
<td>47.7±4.05</td>
<td>48.5±2.31</td>
<td>44.9±3.15(^{ab})</td>
<td>44.9±3.99(^{ab})</td>
<td>43.34±2.96(^{ac})</td>
<td>44.57±1.68(^{bc})</td>
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<tr>
<td>MUFA</td>
<td>17.0±1.27</td>
<td>14.6±0.82(^{aa})</td>
<td>12.8±1.05(^{ab})</td>
<td>12.7±1.50(^{bc})</td>
<td>12.36±0.91(^{ac})</td>
<td>11.95±1.79(^{bc})</td>
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<tr>
<td>PUFA</td>
<td>35.28±4.28</td>
<td>36.80±2.00</td>
<td>42.17±2.63(^{bb})</td>
<td>42.36±2.29(^{bc})</td>
<td>43.43±4.21(^{bc})</td>
<td>43.47±1.67(^{bc})</td>
</tr>
<tr>
<td>n-6</td>
<td>31.11±3.29</td>
<td>34.35±3.38</td>
<td>39.13±2.42</td>
<td>39.70±2.53(^{b})</td>
<td>40.84±2.79(^{bc})</td>
<td>40.56±1.84(^{bc})</td>
</tr>
<tr>
<td>n-3</td>
<td>4.17±1.21</td>
<td>3.55±0.65(^{a})</td>
<td>3.04±0.73</td>
<td>2.66±0.38(^{b})</td>
<td>3.32±0.47</td>
<td>2.91±0.43(^{a})</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>7.78±1.56</td>
<td>9.89±1.87(^{a})</td>
<td>13.49±3.07</td>
<td>15.23±2.76(^{bb})</td>
<td>16.65±3.20</td>
<td>14.20±2.24(^{a})</td>
</tr>
</tbody>
</table>

Significant difference between AGA and SGA groups, \(^{a}p<0.05, \^{a}p<0.01, \^{a}p<0.001\) at the same time points. Significant difference within AGA and SGA groups at birth compared with 14\(^{th}\) (b) and 28\(^{th}\) (c) day, \(^{b}p<0.05, \^{b}p<0.01, \^{b}p<0.001\), respectively. AGA, appropriate for gestational age; SGA, small for gestational age.
Preterm and full-term infants are able to synthesize LCP only in terms of LCPUFA but their precursors as well. This metabolic activity is active at an earlier gestational age and decreases with advancing development. However, it is not clear yet if the synthesis rate is high enough to respond to the demand of both the general and the brain growth in the neonatal period. The LCPUFA level in plasma PL depends on metabolic elongation and desaturation of precursors but also on dietary intake of LCPUFA. Although we found differences in LA and total PUFA between milk of AGA and SGA mothers after 2 weeks, this study did not show any significant difference in phospholipid FA profile between the two groups of infants. However, after 4 weeks of life, SGA infants had a higher proportion of DTA and DPA and a lower proportion of DHA than AGA infants. These results indicate an impaired conversion of DPA to DHA in SGA infants which is in accordance with Llanos et al. DHA level decreased during the first month in SGA infants which is in accordance with Llanos et al. DHA level decreased during the first month in SGA infants which is in accordance with Llanos et al.

The FA composition of human milk is important not only in terms of LCPUFA but also their precursors as well. Preterm and full-term infants are able to synthesize LCPUFA from corresponding precursors: DHA from ALA and arachidonic acid from LA, during the first days of postnatal life, as demonstrated in studies with stable isotopes. Llanos et al. stated that the synthesis was more active at an earlier gestational age and decreased with advancing development. However, it is not clear yet if the synthesis rate is high enough to respond to the demand of both the general and the brain growth in the neonatal period. The LCPUFA level in plasma PL depends on metabolic elongation and desaturation of precursors but also on dietary intake of LCPUFA. Although we found differences in LA and total PUFA between milk of AGA and SGA mothers after 2 weeks, this study did not show any significant difference in phospholipid FA profile between the two groups of infants. However, after 4 weeks of life, SGA infants had a higher proportion of DTA and DPA and a lower proportion of DHA than AGA infants. These results indicate an impaired conversion of DPA to DHA in SGA infants which is in accordance with Llanos et al. DHA level decreased during the first month in SGA infants which is in accordance with Llanos et al. DHA level decreased during the first month in SGA infants which is in accordance with Llanos et al. Our study showed significant differences in serum FA profile at birth between SGA and AGA mildly preterm infants born around 35 gestational weeks, suggesting an altered metabolic transfer of FA via placenta in AGA and SGA pregnancy. Furthermore, we demonstrated different FA profile of serum PL between AGA and SGA infants during first 4 weeks of postnatal life, indicating that SGA infants may have lower metabolic activity than AGA infants of the same gestational age. In addition, FA composition of human milk showed surprisingly low level of n-3.
FA, probably due to a very low dietary intake, which will have an important influence upon the long-term outcome of the physical, intellectual, and visual functions of their children. Taking into account the obtained results, dietary intervention and/or n-3 PUFA supplementation of pregnant and lactating women in our region should be considered.

Source of funding
This work was supported by the Project 41030 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

Conflict of interest
The authors declare no conflict of interest.

References


