Plasma or red blood cell phospholipids can be used to assess docosahexaenoic acid status in women during pregnancy

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Abstract

The suitability of using plasma phospholipids (PLs) to assess docosahexaenoic acid (DHA) status during pregnancy is well accepted. Recent discussions have centered around whether red blood cells (RBCs) can be used to indicate DHA status. We tested the hypothesis that in pregnant women participating in an intervention study when fed a functional food containing DHA, maternal plasma PL DHA would be positively associated with maternal RBC PL and umbilical cord blood RBC PL DHA. Maternal and umbilical cord blood samples were obtained at delivery from women whose mean dietary intake was 187 mg/d (including the amount consumed from the DHA-functional food). Maternal plasma and RBCs and cord blood RBC lipids were extracted and PLs separated by thin-layer chromatography. Phospholipid lipids were methylated, and fatty acids were identified using gas chromatography. Fifty-nine maternal samples and 30 cord blood samples were analyzed. There were moderate to strong correlations between DHA in all compartments (maternal plasma vs maternal RBC PL DHA weight percent [wt%], \( r = 0.633, P \leq .001 \); maternal plasma vs cord blood RBC PL DHA wt%, \( r = 0.458, P \leq .01 \); maternal RBCs vs cord blood RBC PL DHA wt%, \( r = 0.376, P \leq .01 \)). These results support the practice of using either plasma PLs or RBC PLs to assess maternal and infant DHA status.

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Abbreviations: ARA, arachidonic acid; DHA, docosahexaenoic acid; LC-PUFA, long-chain polyunsaturated fatty acid; PL, phospholipid; RBC, red blood cell; wt%, weight percent.

1. Introduction

It is generally accepted that plasma phospholipid (PL) long-chain polyunsaturated fatty acids (LC-PUFAs) reflect tissue LC-PUFA status. During pregnancy, the LC-PUFAs tend to be depleted as these are preferentially transferred to the developing fetus [1,2]. Of these, DHA (22:6n-3) tends to be low in the diets of pregnant women, whereas arachidonic acid (ARA, 20:4n-6) tends to be adequate [3]. This, combined with its importance to fetal neurodevelopment, has put methods for the determination of DHA status of women during pregnancy at the center of recent discussions among scientists. Fatty acid status has generally been assessed by analyzing plasma PLs [4-11].

We assessed PL fatty acid concentrations of maternal and umbilical cord venous blood obtained from subjects participating in a DHA-functional food intervention trial, described previously [12,13], to test the hypothesis that maternal plasma and red blood cell (RBC) PL DHA concentration would be positively correlated with cord
RBC PL DHA. Our objective was to determine the associations between plasma and RBC PL DHA of pregnant women and RBC PL DHA of the umbilical cord with the aim of establishing compartments that can be used with confidence in future studies for assessing DHA status of pregnant women and infants.

2. Methods and materials

2.1. Subjects and sample collection

Women in this study were followed from mid-pregnancy until delivery. Information regarding study design, recruitment, exclusion criteria, supplementation, and compliance was reported previously [12,13]. Briefly, women recruited between 20 and 24 weeks gestation were randomly assigned to consume either a DHA-functional food bar or placebo bar an average of 5 days per week until delivery. This protocol was approved by the institutional review boards at Hartford Hospital (Hartford, Conn) and the University of Connecticut (Storrs, Conn), and informed consent was obtained from all women at the onset of the study.

Maternal and infant umbilical venous bloods were collected at delivery into EDTA-containing tubes. Plasma and RBCs were separated, portioned into aliquots, and stored at −80°C until analyses. Methods for separation, methylation, and identification of plasma and RBC PLs have been described previously [14,15]. Briefly, maternal and cord RBC PLs were extracted according to Folch et al [16]. Maternal plasma lipids were extracted using a modified Folch procedure with 2:1 (vol/vol) dichloromethane/methanol [14] and PLs separated by thin-layer chromatography. Phopholipids were methylated and fatty acids were identified using gas chromatography.

2.2. Statistical analyses

Pearson correlations were performed to determine associations between variables. P values less than .05 were considered significant. There were strong correlations between plasma and RBC LC-PUFAs of the DHA intervention and placebo groups, and group means were not significantly different; therefore, all subjects were grouped together for analyses. Data are presented as means ± SEM. Statistical analyses were performed using SPSS statistical software (version 14.0, Chicago, Ill).

3. Results

Blood samples were collected from 59 mothers and 30 infants. Table 1 provides mean plasma and RBC PL fatty acid concentrations (weight percent [wt%] and mg/L). There were moderate to strong significant correlations for DHA in PLs between all compartments (Fig. 1A-C). Maternal plasma PL and RBC PL ARA were moderately correlated (Fig. 1D), although there were no associations for ARA between maternal and cord blood (Fig. 1E, F). The associations for linoleic acid (18:2n-6) were moderate (cord RBC PLs vs maternal plasma PLs, r = 0.357, P = .049; cord RBC PLs vs maternal RBC PLs, r = 0.498, P = .005; maternal plasma PLs vs maternal RBC PLs, r = 0.316, P = .032). Maternal plasma PL and RBC PL α-linolenic acid (18:3n-3) wt% was not associated, and cord blood α-linolenic acid was not detected. DHA/ARA was positively associated in all compartments (maternal plasma PLs vs cord RBC PLs, r = 0.352, P = .052; maternal RBC PLs vs cord RBC PLs, r = 0.399, P = .029; maternal plasma PLs vs RBC PLs, r = 0.797, P = .000). Mean dietary intake of DHA across all subjects, including intake from the DHA-functional food, was 187 mg/d (without the bar, 79 mg/d) and mean intake of ARA was 153 mg/d.

4. Discussion

Our results support the idea that either plasma PL or RBC PL DHA may be used to assess maternal or infant DHA status. Furthermore, because the bulk of the LC-PUFAs in

<table>
<thead>
<tr>
<th></th>
<th>Maternal plasma PLs</th>
<th>Maternal RBC PLs</th>
<th>Umbilical cord RBC PLs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>wt%</td>
<td>Concentration (mg/L)</td>
<td>wt%</td>
</tr>
<tr>
<td>16:0</td>
<td>34.83 ± 0.36</td>
<td>269.96 ± 9.86</td>
<td>25.66 ± 0.44</td>
</tr>
<tr>
<td>16:1</td>
<td>1.90 ± 0.10</td>
<td>14.24 ± 0.86</td>
<td>1.57 ± 0.09</td>
</tr>
<tr>
<td>18:0</td>
<td>13.15 ± 0.30</td>
<td>99.86 ± 3.39</td>
<td>11.78 ± 0.25</td>
</tr>
<tr>
<td>18:1</td>
<td>14.01 ± 0.51</td>
<td>105.33 ± 5.03</td>
<td>21.76 ± 0.19</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>23.01 ± 0.45</td>
<td>179.89 ± 7.27</td>
<td>13.17 ± 0.29</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>0.06 ± 0.01</td>
<td>0.41 ± 0.09</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>18:3n-4</td>
<td>0.20 ± 0.03</td>
<td>1.64 ± 0.22</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>20:1</td>
<td>0.12 ± 0.03</td>
<td>0.83 ± 0.13</td>
<td>0.43 ± 0.10</td>
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<tr>
<td>20:2</td>
<td>0.27 ± 0.03</td>
<td>2.37 ± 0.26</td>
<td>0.47 ± 0.07</td>
</tr>
<tr>
<td>20:4</td>
<td>9.12 ± 0.30</td>
<td>72.91 ± 3.72</td>
<td>18.23 ± 0.37</td>
</tr>
<tr>
<td>20:5</td>
<td>0.20 ± 0.11</td>
<td>1.49 ± 0.71</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>22:5</td>
<td>0.26 ± 0.04</td>
<td>2.41 ± 0.34</td>
<td>1.88 ± 0.10</td>
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<tr>
<td>22:6n-3</td>
<td>2.87 ± 0.19</td>
<td>22.67 ± 1.59</td>
<td>4.75 ± 0.24</td>
</tr>
</tbody>
</table>
Fig. 1. Scatter plots showing correlations between maternal plasma, RBC, and umbilical cord RBC PL DHA and ARA wt%. Pearson correlations between (A) maternal plasma PL DHA wt% and RBC PL DHA wt% ($r = 0.633, P < .01$), (B) maternal RBC PL DHA wt% and cord RBC PL DHA wt% ($r = 0.390, P < .05$), (C) maternal plasma PL DHA wt% and cord RBC PL DHA wt% ($r = 0.458, P < .01$), (D) maternal plasma PL ARA wt% and RBC PL ARA wt% ($r = 0.376, P < .01$), (E) maternal RBC PL ARA wt% and cord RBC PL ARA wt% ($r = 0.196, P > .05$), (F) maternal plasma PL ARA wt% and cord RBC PL ARA wt% ($r = 0.176, P > .05$).
RBCs are in the PLs, the step of separating the RBC PL class is not necessary; and we suggest that RBC can confidently be used for this assessment. It is well known that plasma PL DHA concentration is indicative of short-term intake and that RBC PL DHA is indicative of longer-term dietary intake. However, dietary intake of DHA does not typically vary over time; thus, plasma or RBC PLs should both be indicative of maternal DHA status.

These results are corroborated by data from several earlier reports in which DHA status was examined in pregnant women. Previous research from our laboratory [15], in a population of nonsupplemented women with socioeconomic status and ethnicity similar to this population, documented the relationship between maternal plasma PL and cord RBC PL DHA. In a cod liver oil supplementation study in the Netherlands, correlations between maternal plasma and RBC PL DHA concentrations at delivery, between cord blood plasma and RBC PL DHA concentrations, and for maternal plasma and RBCs and cord RBC PL DHA have been reported [4]. However, the population of women in that study had significantly higher habitual intakes of n-3 LC-PUFAs compared to our population of women. Moderate correlations between maternal and fetal PL DHA in both plasma and RBCs were documented for a DHA egg supplementation study in young African American women [11]. The DHA eggs provided lower amounts of weekly dietary DHA with a population whose habitual DHA intake was similar to our women. Our current report adds to what is known about the suitability of RBCs for assessing n-3 LC-PUFA status in other populations of women with various ethnicities and different habitual dietary n-3 LC-PUFA intakes. In the study reported here, we looked at a population of mainly white women with low dietary intakes of DHA.

Our results are for a group of pregnant women who participated in a DHA-functional food intervention trial for whom we observed no difference between the intervention and placebo groups with respect to plasma PL DHA concentrations. Others who have examined the impact of moderate DHA supplementation (184 and 200 mg/d as either functional food or supplement) also demonstrated no effect on PL DHA concentration [5,11]. Higher amounts of supplementation, 570 to 1000 mg of DHA, are reflected in PL DHA concentration [4,7,8,17,18]. Thus, our finding is in line with what has previously been reported.

A limitation of this research is that we did not have a greater range of DHA status to establish unequivocally that the relationships we are describing exist across a larger range of intakes and status. Future investigations should include a larger study group.

Recent discussions among scientists who study LC-PUFA status clearly revealed that different research groups use different lipid compartments for assessing LC-PUFA status. The associations we demonstrate herein between plasma and RBC PL DHA lead us to conclude and suggest that RBCs, without the laborious task of separating the PLs, can be used to assess DHA status. The current report adds data from one more population of pregnant women and infants and demonstrates that researchers can have confidence in using either plasma or RBC PLs for establishing DHA status.

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References


