Comparison of breast milk fatty acid composition from mothers of premature infants of three countries using novel dried milk spot technology

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Highlights

- We describe a Dried Milk Spot (DMS) method based on the PUFAcoat™ technology for breast milk fatty acid profiling using gas chromatography.
- This method provides precise and robust results with conventional liquid/liquid extraction method.
- This method was applied in large cross-country clinical trials to demonstrate its sensitivity and applicability in finding small differences in the breast milk fatty acid profiles of women with known different dietary intake.
- This is an easy and inexpensive tool that can be utilised in population screening and large clinical trials.
Comparison of breast milk fatty acid composition from mothers of premature infants of three countries using novel dried milk spot technology

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Summary

Accurate measurements of breast milk fatty acid profile is important to define the dietary fatty acid intake of breastfed infants, and thus to understand the association between level of long chain polyunsaturated fatty acids (LCPUFA) intakes and their potential benefits. We report this dried milk spot (DMS) for breast milk fatty acid profiling using gas chromatography, which overcomes cold chain difficulties involved in the conventional method and eases the analytical process. This method provides precise results for all reported fatty acids, especially for the n-6 and n-3 LCPUFA that have many known clinically important roles in infant health. We have also demonstrated the sensitivity of this DMS method in differentiating the breast milk fatty acid profile of Australian and Singaporean women that are known to have different dietary intakes. The DMS method report in this study can be applied to clinical trials and population studies.
Abstract

Long chain polyunsaturated fatty acid (LCPUFA) intake during infancy has been associated with many health benefits, and the LCPUFA intake of breastfed infants is largely dependent on the composition of breast milk. The conventional method for breast milk fatty acid profiling is complicated by the need for cold-chain transportation and storage, and the newly developed dried milk spot (DMS) technology overcomes these difficulties. This study aimed to determine the accuracy, sensitivity and applicability of the DMS method developed based on the PUFACoat™ technology. Two hundred breast milk samples were analyzed using the conventional method and compared with the DMS method. In order to evaluate the usefulness of DMS for large scale international studies, we analyzed another 786 breast milk samples collected from mothers of preterm infants who participated in a large clinical trial conducted in Australia, New Zealand and Singapore. Fatty acids were measured using capillary gas chromatography and results were reported as weight percentage of total fatty acids. Strong correlations and tight variation were observed in total saturated, monounsaturated, n-6 and n-3 PUFAs between the conventional and DMS methods. The DMS method proved to be sensitive in differentiating the breast milk fatty acid profiles of women consuming different habitual diets as evidenced by the differences between the breast milk fatty acid composition between Australian and Singaporean population. This study demonstrates that the DMS and the conventional method provide interchangeable results, and the DMS method is a particularly useful tool for large-scale studies.

Key Words: dried milk spot, breast milk lipids, long-chain polyunsaturated fatty acids, analytical method comparison
Abbreviations: DMS: dried milk spot; LCPUFA: long chain polyunsaturated fatty acid; LA: linoleic acid; EPA: Eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; FAME: fatty acid methyl ester

1 Introduction

The level of LCPUFA intake during infancy and their potential health benefits continue to be studied because of evidence suggesting benefits with respect to visual acuity [1], immune responses [2] and reduced risks of allergic diseases [3, 4], that may persist through childhood. Dietary LCPUFA intake of breastfed infants is mainly dependent on breast milk level, which in turn is largely defined by maternal intake. Lactating mothers with differing dietary intakes have distinct breast milk fatty acid profiles, especially the n-6 and n-3 PUFAs [5]. Accurate measurements of breast milk fatty acid composition helps to better define the fatty acid intake of the breastfed infant and to better understand the roles of fatty acids during infancy. The conventional methods for breast milk fatty acid profiling requires liquid breast milk samples and strict temperature control during storage and transportation, which increases the cost of clinical trials and so limits the ability of assessing breast milk fatty acid profile in large multi-centre studies. An easy and inexpensive tool for collecting and analysing the breast milk fatty acid composition would clearly be of value.

One such technique has been developed based on the current dried blood technology [6, 7], namely the dried milk spot (DMS) technology, which has been commercialized [8, 9]. The method only requires a single drop of breast milk, simplifies the analytical process, and does not require cold-chain transportation and storage. Our group have developed a dried blood spot collection system (PUFAcoat™) [10] used silica gel impregnated paper in combination with an antioxidant and a chelating agent, which can potentially be used as a DMS method. The stability of fatty acid composition of breast milk spotted on the paper was shown to be stable at room temperature for at least four weeks [10].
Therefore, the aims of the study were to compare the DMS method based on the PUFAcoat™ technology against the conventional method for breast milk fatty acid profiling; and to apply this DMS method in a large clinical trial to determine its sensitivity in differentiating the breast milk fatty acid profiles of women with different habitual diets.

2 Materials and methods

2.1 Method comparison

2.1.1 Subjects and sampling

Stored breast milk samples from women who participated in the PINK (Pregnancy Iodine and Neurodevelopment in Kids) study were used for method comparison. The PINK study assessed the relationship between the maternal iodine status during pregnancy and the neurodevelopmental outcomes of infants [11-13]. Briefly, women (n=784) were recruited from Women’s and Children’s hospital and Flinders Medical Centre, South Australia, between 2011 and 2012. Breast milk samples were collected either at the hospital during a clinic visit or at the participants’ home at 3 month postpartum for iodine concentration measurement. Between 5 and 9 am of the collection day, foremilk (10~15ml) was collected before the first feed using a provided container (70ml sterilised pot, Southern Cross Scientific Ltd, South Australia), and samples were kept in the home or hospital freezer and transferred to the laboratory and stored at freezer (-80°C) within an average of 9 days of collection. Two hundred women who consented their surplus samples to be used for further analysis were randomly selected for this current study.

The study was approved by Women’s and Children’s Hospital Human Research Ethic Committee (HREC) in South Australia. Each breast milk sample was analyzed by both a conventional liquid/liquid extraction method [14] and the DMS method for total fatty acid profiling. The DMS collection cards were prepared in bulk 1-2 months in advance, were
packed in foil bags with desiccants and stored at room temperature (~25°C) prior to use. Milk samples were thawed and then aliquoted onto the collection cards at the same time of initiating the conventional method. The chemical analysis of both methods were conducted at the same time.

2.1.2 DMS system preparation

The procedures of DMS spot card preparation were based on the PUFAcoat™ method described previously [10]. Briefly, Whatman ion exchange papers (Grade SG81, Whatman, Buckingham, UK) were cut to desired size and then coated with antioxidant butylated hydroxytoluene (Sigma-Aldrich, St Louis, MO) at concentration of 2mg/ml and chelating agent ethylenediaminetetraacetic acid (Chem-supply, Gillman, Australia) at concentration of 5mg/ml in 70% ethanol; the papers were then dried and packed in pre-manufactured cards.

To prepare DMS samples in the laboratory, 20µL of thawed breast milk was spotted onto the collection paper, and left to dry at room temperature for at least three hours.

2.2 Application of DMS method

2.2.1 Subject and sampling (international study)

Breast milk samples collected by women, whose very preterm infants (<29 weeks gestational age at birth) participated in the N3RO (N-3 fatty acids for improvement in Respiratory Outcomes) randomised controlled trial [15, 16], were utilised to test the utility of the DMS method in this study. The mother-infant pairs were recruited from 13 centers around Australia, New Zealand and Singapore, where the differences in diet between countries are expected to produce differences in breast milk fatty acid profiles. Details of the recruitment were reported elsewhere [15, 16]. Among 1098 mothers involved in the trial, 736 of them provided breast milk for fatty acid analysis and were included in this current study. The study protocol was approved by the relevant HREC (Australia: South Adelaide Clinical HREC;
Women’s and Children’s Health Network HREC; The Royal Women’s Hospital HREC; The Southern Health HREC; Mercy Health HREC; Women and Newborn Health HREC; Hunter New England HREC. New Zealand: Northern B Health and Disability HREC. Singapore: SingHealth Centralised Institutional Review Board E).

At the time of discharge from the neonatal unit (36 week of postmenstrual age), each mother spotted breast milk onto a DMS card, which was left to dry at room temperature for at least three hours prior to transporting to a central laboratory. All samples were stored in foil bags containing a desiccant and transported through the Post (Australia) and FedEx (Singapore and New Zealand). Samples were then stored at -20°C immediately after arrival and were analyzed within 4 weeks of arrival.

2.2.2 Methods

2.3 Fatty acids analysis

2.3.1 Conventional method

Breast milk samples were thawed at room temperature in a cardboard box to avoid direct sunlight, and then shaken vigorously before sampling. The breast milk fats were extracted according to a modified Folch method [14]. In brief, 200µL of breast milk was extracted with chloroform and methanol (2:1 v/v) and the chloroform layer containing all lipids was evaporated under a nitrogen stream. Dried lipids were then redissolved in 300µL 9:1 chloroform: methanol and 60µL of this solvent containing the lipid extract was used for fatty acid determination. Fatty acids were transmethylated to fatty acid methyl ester (FAME) using methanol with 1% sulphuric acid as a catalyst for three hours at 70°C [10, 17]. FAME were then extracted into heptane for analysis by gas chromatography.
2.3.2 **DMS method**

Milk spots were excised using cleaned scissors and tweezers, and were then placed in transmethylation fluid and treated as described above. The resulting FAME were extracted and analyzed by gas chromatography.

2.3.3 **Gas chromatography**

The analysis of FAME were carried out using a Hewlett-Packard 6890 (Hewlett-Packard, CA, USA) gas chromatograph, which was equipped with a vaporization injector, a flame ionization detector, and a BPX70 capillary column 50 m x 0.32 mm, film thickness 0.25µm (SGE Pty Ltd., Victoria, Australia). The temperature of the injector and detector were set as 250°C and 300°C, respectively. The carrier gas was Helium with a flow rate of 1 ml/min in the column and the inlet split ratio was set at 20:1. The fatty acids in samples were identified based on the retention times and peak area values of the commercial FAME standards (Nu Chek Prep Inc., Elysian, USA).

2.4 **Statistical analysis**

Fatty acid data are expressed as weight percentage of total fatty acids and presented as median with interquartile range (IQR) since the majority of the data were not normally distributed. The correlation between DMS and conventional method of individual fatty acids results was assessed using Spearman’s order-rank correlation. Bland-Altman test, which is defined as the ratio of fatty acid content from two methods (DMS vs. conventional) against the average results of the two methods, was carried out to assess the agreement between the DMS and the conventional method. The difference in breast milk fatty acid composition of women from any two of the three countries were assessed using Mann-Whitney U test. Statistical analyzes were performed using the SPSS (Version 23.0, IBM Corp, in Armonk, NY) and GraphPad Prism (Version 6 v008, for Windows, GraphPad software, La Jolla California USA) and the statistical significant level was set to be 0.05.
3 Results

3.1 Method comparison

The Spearman correlation between the results obtained from DMS and conventional method are presented in Table 1. The correlation for most of reported fatty acids were high (R>0.95) with exception of fatty acids C10:0, trans C16:1, trans 18:2 and C20:5 n-3 (eicosapentaenoic acid EPA); the correlations for these four fatty acids were 0.7212, 0.5651, 0.8026 and 0.8467, respectively. The association between breast milk total saturates, monounsaturates, n-6 and n-3 PUFA analyzed using DMS and conventional method are shown in Figure 1a-1d and demonstrated a strong correlation between the two methods (R=0.9981, 0.9958, 0.9983 and 0.9892, respectively; p<0.0001 for all).

The results of the Bland-Altman test are presented in Figure 2a-2d, the mean ratio (95% limits of agreement) of two methods (DMS vs. Folch) was 1.00 (0.99-1.02) for total saturates, 1.00 (0.98-1.01) for total monounsaturated, 0.99 (0.96-1.01) for total n-6 PUFA and 0.97 (0.91-1.04) for total n-3 PUFA.

3.2 Application of DMS method

The breast milk fatty acid profile of Australian, New Zealand and Singaporean mothers using DMS are presented in Table 2 along with the differences in the profile between any two of the three populations.

Differences in fatty acid profile between Australian and Singaporean women were seen in the majority of the fatty acids except total saturates (p=0.142), total n-3 PUFA (p=0.719), EPA (p=0.052) and n-3 docosapentaenoic acid (p=0.061). Breast milk of New Zealand women were significantly higher than Australian women in percentage of total saturates (p=0.001), total monounsaturates, total trans and total n-3 PUFA (p<0.0001), but lower in percentage of total n-6, linoleic acid (LA), arachidonic acid (AA) and docosahexaenoic acid.
(DHA) (p<0.0001). When comparing Australian women with New Zealand women, the breast milk of Australian women was lower in the percentage of total saturates (p=0.010), total trans (p<0.0001), but higher in the percentage total n-6 PUFA including LA (p=0.002) and AA (p=0.003). The breast milk n-3 PUFA levels were similar between the two populations, with the exception of the percentage of DHA being higher in the breast milk from Australian women compared to that of New Zealand women (p=0.008).

4 Discussion

The Bland-Altman test showed narrow limits of agreements between the results obtained from the conventional and the DMS method across a range of concentrations for different types of fatty acids. Variations of most samples are within 5% for total saturates, monounsaturates and n-6 PUFA. Slightly greater variation was observed in total n-3 PUFA as these fatty acids only presented in human breast milk in very small quantities, which is more likely to result in greater variations. However, the variation for the vast majority of n-3 PUFAs are within 10%, and only 6 out of 200 samples were greater than 10% but less than 20% variation, which was an acceptable range [18]. Strong correlations between the conventional and DMS methods for n-6 and n-3 PUFA makes the method particularly attractive because of the known clinical importance of these compounds for infants.

The DMS method described in this study allows the measurements of breast milk fatty acid composition more accurately with even less sample required than in the studies published previously [8, 9]. The current DMS has advantages compared to the methodologies previously published, as it consists of three protecting components that have been proven to be necessary to stabilise LCPUFA [10]. The filter paper used in this study was silica gel impregnated, which allows stronger binding with the lipids than the non-silica coated Ahlstrom (Grade 226) filter paper used in previous studies [8, 9], and the addition of chelating agent that used in our PUFAcoat™ system offers more protection from oxidation.
The DMS collection card can be prepared in advance of sample collection, as it has been demonstrated to be stable at room temperature for at least two months [19].

A major aim of this study was to utilise DMS method to measure the breast milk fatty acid profiles of populations with known differences in their habitual diets, and hence their breast milk fatty acid profiles. The differences found in breast milk fatty acid profile between Australian and Singaporean women are in line with the existing evidence that the South East Asian populations that eat more fishes that are rich in omega-3 fatty acids [5]. This demonstrated the sensitivity of DMS method in differentiating breast milk fatty acid profiles of women with different dietary habits. Interestingly, the breast milk profiles of Australian and New Zealand women, which are thought to have similar dietary habits, differed in total saturates, total trans fats, n-6 PUFA and DHA content. This might be due to a larger percentage of indigenous participants included in the New Zealand cohort (21% New Zealand vs. 5% in Australia), the diet of whom may be different from Caucasian women in New Zealand or Australia. However, there are no existing data on breast milk profile of New Zealand population and no dietary information was collected in the N3RO trial to further confirm whether the results are actually a reflection of difference in dietary intake. In addition, the sample size of New Zealand cohort is relatively small, which may not be a true representation of the New Zealand population.

4.1 Strengths and limitations

We established the strong positive linear relationship between the DMS and conventional methods in a large sample (N=200) over the wide range of fatty acids seen in clinical practices. Thus the DMS method provides robust results compared with conventional liquid/liquid extraction method for breast milk fat and fatty acid profiling. The use of DMS method for breast milk fatty acid analysis also overcomes the difficulties implicit in conventional methods, including the need for temperature regulations during storage and
transportation. Thus samples can be transported by standard postal service from the test site to the laboratory for analysis. Because DMS can be transmethylated for GC directly without extraction, the method also simplifies the laboratory procedure, reduces costs and allows increased throughput, making it an attractive option for use in population screening and large clinical trials. However, the current method is limited in the ability to measure the absolute amount of fat in any breast milk sample unless a known volume of breast milk is spotted on the DMS paper. The variations in the absolute fatty acid concentration between DSM and the conventional methods remain to be investigated in further studies. The applicability of method was demonstrated using samples from a multi-centre international clinical trials where dietary differences were expected to be both marked (Singapore vs. Australia) and subtle (Australia vs. New Zealand). Though the stability of breast milk fatty acid was not tested in this study, we have previously demonstrated that the fatty acid composition in biological fluids are stable at room temperature for weeks [10].

5 Conclusion

Highly correlated results between DMS and a conventional method for determination of breast milk fatty acids were demonstrated for both accuracy and precision. Using the DMS method, differences were found in fatty acid composition of breast milk between Australian and Singaporean that are consistent with known differences in diet, which support the sensitivity and applicability of the method. This simple, precise and high throughput DMS method is suited for large population studies.

Acknowledgement

This work was funded by the NHMRC of Centre of Research Excellence Grant (1035530). CG received Adelaide Graduate Research Scholarship from the University of
Adelaide. RAG was supported by NHMRC Senior Research Fellowship (APP 1046207). MM was supported by NHMRC Principal Research Fellowship (APP1061704).

**Author contributions**

GL, RAG, AJM, SJZ, CTC, MM and JM designed the concept of the study. CG conducted the experiments and analyzed the data. All authors contributed to the interpretation of the results, drafting of the manuscript and approved the final version to be submitted for publication.

**Conflict of interest**

RAG and MM have received honoraria for scientific advisory board contributions to Fonterra. All honoraria are paid to the Healthy Mothers, Babies and Children, South Australian Health and Medical Research Institute to support continuing education activities for students and postgraduates. RAG and GL are the inventor of PUFAcoat™ system, the patent of which is owned by the University of Adelaide. The licence for PUFAcoat™ has been granted to Xerion Ltd Melbourne. Other authors declare no conflict of interest.

**References**

6. A.H. Metherel, R.C. Hogg, L.M. Buzikievich et al., Butylated hydroxytoluene can protect polyunsaturated fatty acids in dried blood spots from degradation for up to 8 weeks at room temperature, Lipids Health Dis 12 (2013) 22.


Figure 1a-1d. Correlation of breast milk total saturates (1a.), total monounsaturates (1b.), total n-6 (1c.) and n-3 (1d.) fatty acid analysis between conventional and dried milk spot methods (N=200). FA%: weight percentage of total fatty acids; PUFA: polyunsaturated fatty acid; DMS: dried milk spot.
Figure 2a-2d. Bland-Altman plots comparing results of conventional Folch method and Dried Milk spot (DMS) method for total saturated (2a.), total monounsaturated (2b.), total n-6 (2c.) and n-3 (2d.) fatty acid analysis showing the ratio vs mean results from two methods. The solid line represents the mean ratio and the dotted line represents 95% limits of agreement (N=200). FA%: weight percentage of total fatty acids; PUFA: polyunsaturated fatty acid; DMS: dried milk spot.
### Tables

Table 1. Spearman correlation between conventional and Dried Milk Spot (DMS) method for fatty acid analysis (weight% of total fatty acids) (N=200)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Method</th>
<th>Conventional</th>
<th>DMS</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Saturates</strong></td>
<td></td>
<td>43.81 (39.85, 47.04)</td>
<td>43.77 (39.96, 46.90)</td>
<td>0.9981</td>
</tr>
<tr>
<td>10:0</td>
<td></td>
<td>0.91 (0.78, 1.05)</td>
<td>1.08 (0.89, 1.27)</td>
<td>0.7212</td>
</tr>
<tr>
<td>12:0</td>
<td></td>
<td>4.94 (3.93, 5.98)</td>
<td>4.90 (3.84, 5.80)</td>
<td>0.9777</td>
</tr>
<tr>
<td>14:0</td>
<td></td>
<td>6.18 (5.24, 7.49)</td>
<td>6.09 (5.14, 7.33)</td>
<td>0.9933</td>
</tr>
<tr>
<td>15:0</td>
<td></td>
<td>0.36 (0.29, 0.44)</td>
<td>0.36 (0.29, 0.44)</td>
<td>0.9809</td>
</tr>
<tr>
<td>16:0</td>
<td></td>
<td>23.11 (21.22, 24.76)</td>
<td>23.08 (21.22, 24.73)</td>
<td>0.9894</td>
</tr>
<tr>
<td>18:0</td>
<td></td>
<td>7.11 (5.91, 8.21)</td>
<td>7.21 (6.06, 8.27)</td>
<td>0.9971</td>
</tr>
<tr>
<td><strong>Total Trans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T16:1</td>
<td></td>
<td>1.21 (0.89, 1.52)</td>
<td>1.23 (0.92, 1.51)</td>
<td>0.9589</td>
</tr>
<tr>
<td>T18:1 n-9</td>
<td></td>
<td>0.22 (0.17, 0.26)</td>
<td>0.23 (0.18, 0.27)</td>
<td>0.9348</td>
</tr>
<tr>
<td>T18:1 n-7</td>
<td></td>
<td>0.75 (0.51, 0.94)</td>
<td>0.74 (0.52, 0.95)</td>
<td>0.9701</td>
</tr>
<tr>
<td>T 18:2</td>
<td></td>
<td>0.14 (0.11, 0.19)</td>
<td>0.14 (0.11, 0.18)</td>
<td>0.8026</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>40.25 (38.32, 42.69)</td>
<td>40.16 (38.32, 42.59)</td>
<td>0.9958</td>
</tr>
<tr>
<td><strong>Monounsaturates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16:1 n-7</td>
<td></td>
<td>2.31 (1.92, 2.82)</td>
<td>2.30 (1.89, 2.77)</td>
<td>0.9967</td>
</tr>
<tr>
<td>18:1 n-9</td>
<td></td>
<td>35.64 (33.38, 38.03)</td>
<td>35.38 (33.51, 37.99)</td>
<td>0.9945</td>
</tr>
<tr>
<td>18:1 n-7</td>
<td></td>
<td>1.80 (1.60, 2.01)</td>
<td>1.79 (1.59, 1.99)</td>
<td>0.9387</td>
</tr>
<tr>
<td>Total n-9</td>
<td></td>
<td>36.07 (33.86, 38.46)</td>
<td>35.89 (34.00, 38.50)</td>
<td>0.9947</td>
</tr>
<tr>
<td>Total n-7</td>
<td></td>
<td>4.20 (3.54, 4.79)</td>
<td>4.14 (3.51, 4.76)</td>
<td>0.9885</td>
</tr>
<tr>
<td><strong>Total n-6 PUFA</strong></td>
<td></td>
<td>11.76 (9.87, 14.71)</td>
<td>11.51 (9.70, 14.48)</td>
<td>0.9983</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td></td>
<td>10.66 (8.75, 13.55)</td>
<td>10.47 (8.64, 13.34)</td>
<td>0.9978</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td></td>
<td>0.38 (0.32, 0.44)</td>
<td>0.38 (0.31, 0.44)</td>
<td>0.9648</td>
</tr>
<tr>
<td><strong>Total n-3 PUFA</strong></td>
<td></td>
<td>1.51 (1.26, 1.86)</td>
<td>1.48 (1.23, 1.77)</td>
<td>0.9892</td>
</tr>
<tr>
<td>18:3 n-3</td>
<td></td>
<td>0.95 (0.79, 1.23)</td>
<td>0.93 (0.76, 1.18)</td>
<td>0.9842</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td></td>
<td>0.08 (0.06, 0.11)</td>
<td>0.08 (0.05, 0.10)</td>
<td>0.8467</td>
</tr>
<tr>
<td>22:5 n-3</td>
<td></td>
<td>0.17 (0.14, 0.20)</td>
<td>0.16 (0.14, 0.20)</td>
<td>0.9568</td>
</tr>
<tr>
<td>22:6 n-3</td>
<td></td>
<td>0.23 (0.17, 0.34)</td>
<td>0.23 (0.17, 0.33)</td>
<td>0.9953</td>
</tr>
</tbody>
</table>

PUFA: polyunsaturated fatty acids

Data are presented as median (interquartile range)
Table 2. Breast milk fatty acid composition of women from Australia, New Zealand and Singapore using the Dried Milk Spot (DMS) method (weight% of total fatty acid) (N=736)

<table>
<thead>
<tr>
<th></th>
<th>Australian women</th>
<th>New Zealand women</th>
<th>Singaporean women</th>
<th>p</th>
<th>Australian vs. New Zealand</th>
<th>Australia vs. Singapore</th>
<th>New Zealand vs. Singapore</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Saturates</strong></td>
<td>40.9 (37.3-44.3)</td>
<td>42.3 (39.3-45.95)</td>
<td>40.5 (37.3-42.76)</td>
<td>0.010</td>
<td>0.142</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Total Monounsaturated</strong></td>
<td>43.3 (40.3-46.01)</td>
<td>43.0 (40.5-45.36)</td>
<td>40.7 (39.1-42.84)</td>
<td>0.348</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Total Trans</strong></td>
<td>1.15 (0.86-1.49)</td>
<td>1.45 (1.13-1.79)</td>
<td>0.48 (0.27-0.65)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>Total n-6 PUFA</strong></td>
<td>12.2 (10.5-12.94)</td>
<td>11.2 (9.59-13.10)</td>
<td>15.8 (14.5-17.98)</td>
<td>0.001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>18:2 n-6</strong></td>
<td>10.8 (9.28-11.75)</td>
<td>10.0 (8.42-12.65)</td>
<td>14.4 (13.2-16.76)</td>
<td>0.002</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>20:4 n-6</strong></td>
<td>0.42 (0.36-0.48)</td>
<td>0.38 (0.32-0.45)</td>
<td>0.46 (0.38-0.51)</td>
<td>0.003</td>
<td>0.005</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>Total n-3 PUFA</strong></td>
<td>1.75 (1.45-1.95)</td>
<td>1.74 (1.49-1.95)</td>
<td>1.74 (1.43-2.35)</td>
<td>0.439</td>
<td>0.719</td>
<td>0.489</td>
<td></td>
</tr>
<tr>
<td><strong>18:3 n-3</strong></td>
<td>1.14 (0.89-1.44)</td>
<td>1.18 (0.93-1.37)</td>
<td>0.84 (0.67-1.21)</td>
<td>0.923</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td><strong>20:5 n-3</strong></td>
<td>0.40 (0.07-0.12)</td>
<td>0.09 (0.07-0.12)</td>
<td>0.08 (0.06-0.12)</td>
<td>0.266</td>
<td>0.052</td>
<td>0.455</td>
<td></td>
</tr>
<tr>
<td><strong>22:5 n-3</strong></td>
<td>0.19 (0.16-0.23)</td>
<td>0.19 (0.17-0.23)</td>
<td>0.18 (0.14-0.23)</td>
<td>0.563</td>
<td>0.061</td>
<td>0.080</td>
<td></td>
</tr>
<tr>
<td><strong>22:6 n-3</strong></td>
<td>0.26 (0.20-0.27)</td>
<td>0.23 (0.19-0.27)</td>
<td>0.52 (0.39-0.66)</td>
<td>0.008</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

PUFA: polyunsaturated fatty acids

Data are presented as median (interquartile range)