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Research Article

Women from Newfoundland and Labrador Have High Levels of Omega-6 Fatty Acids in Breast Milk Compared to Other Canadian Women: A Cross-Sectional Study

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Abstract:
Omega (n)-3 and n-6 polyunsaturated fatty acids (PUFA) in breast milk and cord blood play a decisive role in proper growth and development. An imbalance in n-6 to n-3 PUFA ratio is associated with several pathological conditions. Newfoundland and Labrador (NL) has the highest rate of obesity and heart disease in Canada; incidences of asthma and allergies are also on the rise in the province. The aim of the present study was to measure the fatty acid composition of breast milk and corresponding cord blood samples of women from NL and to compare that to similar Canadian studies. Breast milk and cord blood samples were collected from 47 women and the relative percentage of fatty acids was measured using gas liquid chromatography. Breast milk arachidonic acid (AA) and docosahexaenoic acid (DHA) levels were 0.77 and 0.45%, while respective cord blood levels were 13.84, 4.64%. A comparison of breast milk AA and DHA levels of women from NL to that of Vancouver and Canada revealed significantly higher levels of AA and DHA. The levels of total PUFA in breast milk and cord blood were 18.88 and 33.18% respectively, and the n-6 to n-3 PUFA ratio was 7.42 and 5.01 respectively. Women from NL show higher levels of AA and DHA in breast milk, along with total n-6 PUFA compared to the other regions of Canada. These findings are important for further research to determine their possible role in the etiology of obesity, metabolic disorders and allergic manifestations in NL population.

Keywords: Arachidonic Acid; Breast Milk; Cord Blood; Docosahexaenoic Acid; Eicosapentaenoic Acid

Introduction: The developing fetus requires an adequate amount of specific fatty acids to support rapid cellular growth and activity. Fatty acids are important biological constituents with metabolic, structural and signaling functions. Omega (n)-3 and n-6 polyunsaturated fatty acids (PUFAs) are the essential fatty acids that are crucial during fatal development [1]. N-3 PUFA such as docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), and arachidonic acid (AA; 20:4), an n-6 PUFA, have gained a significant attention in the recent years due to their importance in fetal development [2] and post birth biological effects. The potential maternal health benefits of n-3 PUFA include prevention of pre-eclampsia [3], prevention and treatment of perinatal depression [4], optimization of infant cognitive and visual development [5], and potential primary prevention of allergic manifestations in infancy and childhood [6]. In addition, various studies showed that diets rich in n-3 PUFA during pregnancy increased birth weight and prolonged pregnancy, thereby reducing the incidence and severity of preterm births and low birth weight infants [7]. This is further confirmed by epidemiological and observational studies, showing an association between a decrease in erythrocyte and plasma DHA and EPA levels with common complications of pregnancy, including preterm birth, pre-eclampsia, and perinatal depression [8].

During pregnancy, the fetus receives its fatty acid supply from the mother via active placental transport from umbilical cord blood, and after birth from breast milk. The levels of PUFA in cord blood and breast milk has been linked with several developmental aspects such as visual development [5], immune function [9] and cognitive abilities [5]. The role of n-3 PUFA during lactation is also equally important; studies have shown that n-3 PUFA in the mother's breast milk or in fortified infant formulas improve neurocognitive and visual development in comparison to infants who received infant formula without DHA [10]. AA, along with EPA and DHA are also required for the fetal and infant's central nervous system growth and development [11]. Embedded A in the cell membrane phospholipids is involved in cell signaling pathways, cell division and serves as an inflammatory precursor for prostanoids and eicosanoids [12].

Newfoundland and Labrador (NL) has the highest rate of obesity [13] and heart disease [14-16] in all of Canada. Incidences for asthma and allergies are also increasing compared to other provinces of Canada [17,18]. Several studies have shown association for these diseases with poor maternal nutrition, especially a diet low in n-3 PUFA [19,20]. However, there is no data available on the n-3 and n-6 PUFA status of breast milk or cord blood of women from NL. The aim of this study was to measure the fatty acid composition of breast milk and corresponding cord blood samples of women from NL and to compare those to similar studies published from other parts of Canada.

Materials and Methods:

Females who attended the breast feeding clinic at the Health Sciences Center, Eastern Health, St. John's, NL, Canada, between September 2011-February 2012, were enrolled. The subjects were healthy and gave natural birth at full term. After written consent, breast milk samples were collected from 50 women within 2 weeks after delivery. The breast milk samples were collected at mid feeding between 2:30-3:30 pm. Corresponding cord blood samples were only available for 47 women, thus the total sample size was n=47.

Sample collection and fatty acid analysis: Mothers of full term babies, who visited the breast feeding clinic within 2 weeks of delivery, were instructed to collect 2-5ml of breast milk samples in clean/sterile tubes, and samples were immediately stored on ice. The corresponding cord blood samples were obtained from the Blood Bank at Health Sciences Centre, Eastern Health, St. John's, NL, Canada. The breast milk and cord blood samples were immediately stored at -80°C for further analysis.

Total lipids were extracted from breast milk and whole cord blood using Folch extraction method [21]. Extracted lipids were transmethylated according to our previously published methods and subjected for gas chromatography analysis [22]. Delta-6 desaturase activities were calculated from the selected fatty acids ratio in breast milk and cord blood total lipids: 20:3n6/18:2n6 for n-6 PUFA and 20:4n3/18:3n3 for n-3 PUFA, as previously described by Peng et al. [23].

Statistical Analysis: All the values were presented as % mean ± SEM (n=47) for fatty acid analysis. One-way ANOVA was performed to compare data from the current study with reported data from Vancouver [24] and Canada [25] using online statistical calculator [26]. Student's t-test was performed for comparing delta-6 desaturase activity using graph pad prism; each bar represents mean ± SEM.

Results:

Breast milk and cord blood fatty acid composition: Breast milk contained 18.88% ± 0.36 of total PUFA, with LA representing 14.30% ± 0.29 (Table 1). A level of breast milk was 0.77% ± 0.03 (Table 1, Figure1A). A comparison of breast milk A of NL women with the values reported for Vancouver [24] and Canada [25] revealed that NL women have significantly higher ($P<0.0001$) levels of breast milk A (Figure 2A). Breast milk DHA level was 0.45% ± 0.03 (Figure 1B), which was also significantly higher compared to Vancouver [24] and Canada [25] ($P<0.0001$) (Figure 2B).

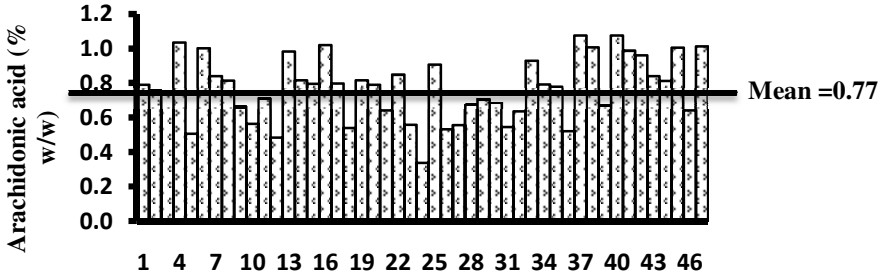

Figure 1A: Individual Subjects

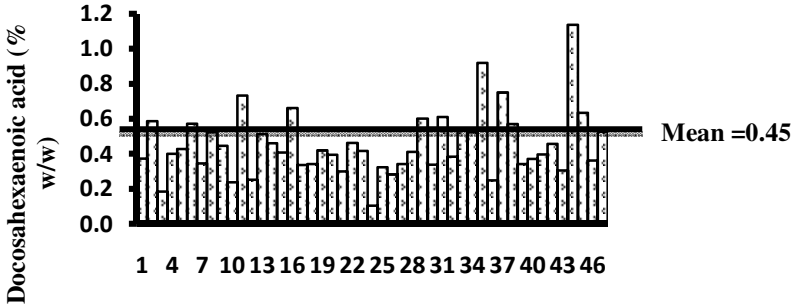

Figure 1B: Individual Subjects

Figure 1: Arachidonic acid (A) and docosahexaenoic acid (B) levels in breast milk of women from Newfoundland and Labrador. Total lipids were extracted and fatty acids were analyzed as described under the methods section. Each bar represents percentage (w/w) arachidonic acid and docosahexaenoic acid in individual subjects; the horizontal line represents the mean distribution of arachidonic acid and docosahexaenoic acid (n=47).

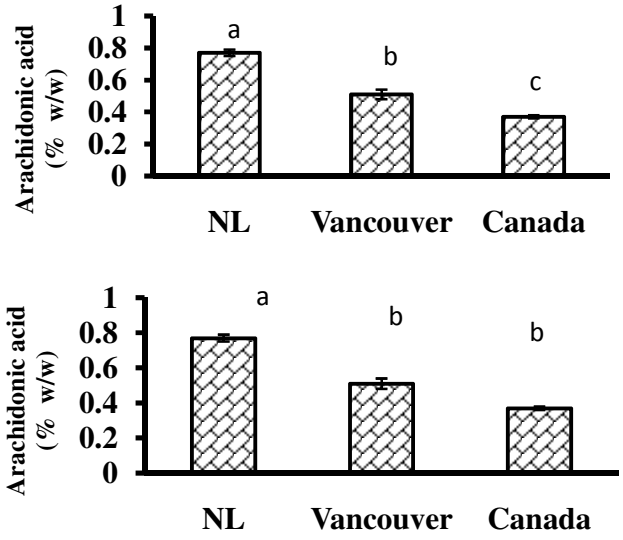

Figure 2: Comparison of breast milk arachidonic acid (A) and docosahexaenoic acid (B) levels of women from Newfoundland and Labrador (NL) with reported data from Vancouver [24] and Canada [25]; details are provided in the manuscript. Each bar represents the percentage (w/w) of fatty acids, mean ± SEM; NL (n=47), Vancouver (n=17) and Canada (n=48). Results were analyzed using One-way analysis of variance (ANOVA) using online statistical calculator. Different superscripts (a,b,c) represent significant differences of $P < 0.05$.

Cord blood contained 33.18% ± 0.41 of total PUFA, with LA representing 7.56% ± 0.24 (Table 2). A level was 13.84% ± 0.24 (Table 2; Figure 3A), whereas DHA level in cord blood was 4.64% ± 0.16 (Table 2; Figure 3B). Moreover, both breast milk and cord blood were rich in palmitic acid (21.5% and 25.86% respectively) and oleic acid (36.39 and 14.53% respectively) (Table1, 2).

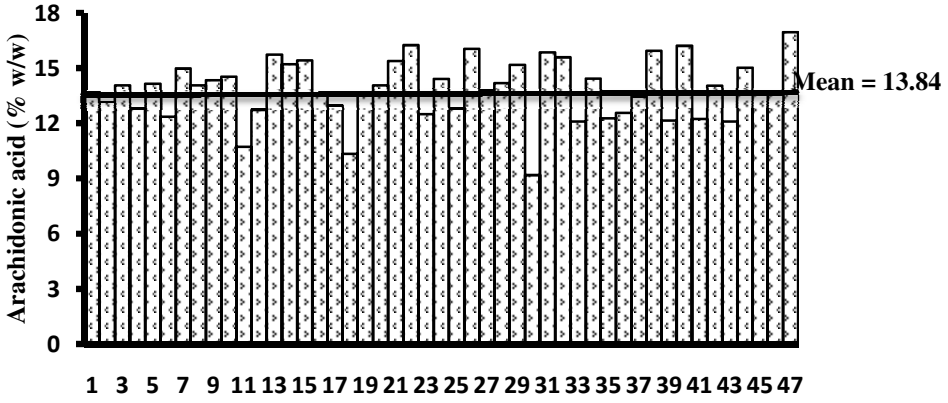

Figure 3A: Individual Subjects

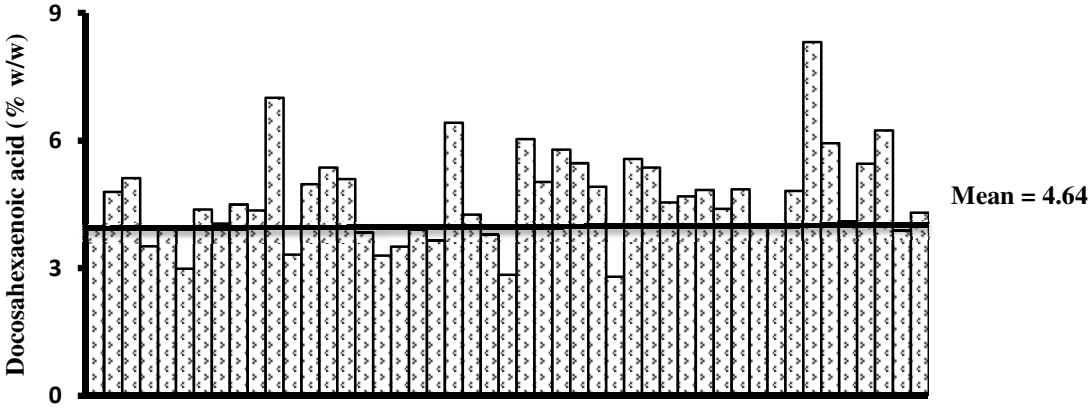

Figure 3B: Individual Subjects

Figure 3: Arachidonic acid (A) and docosahexaenoic acid (B) levels in cord blood of women from Newfoundland and Labrador. Total lipids were extracted and fatty acids were analyzed as described under the methods section. Each bar represents percentage (w/w) of arachidonic acid and docosahexaenoic acid in individual subjects; the horizontal line represents the mean distribution of arachidonic acid and docosahexaenoic acid (n=47).

Fatty Acids (% w/w)	Breast milk
Saturated Fatty acids	
C14:0	6.07 ± 0.26
C15:0	0.32 ± 0.01
C16:0	21.50 ± 0.3
C18:0	6.71 ± 0.24
C20:0	0.19 ± 0.01
C22:0	0.09 ± 0.01
C24:0	0.1 ± 0.01
Total SFA	34.98
Monounsaturated Fatty acids	
C14:1	0.21 ± 0.01
C16:1n-9	0.59 ± 0.04
C16:1n-7	2.87 ± 0.13
C16:1n-5	0.15 ± 0.01
C18:1n-9	36.39 ± 0.47
C18:1n-7	2.43 ± 0.05
C24:1	0.14 ± 0.01
Total MUFA	42.78
Polyunsaturated Fatty acids	
n-6 PUFA	
C18:2n-6	14.30 ± 0.29
C18:3n-6	0.15 ± 0.01
C20:2n-6	0.49 ± 0.03
C20:3n-6	0.65 ± 0.03
C20:4n-6	0.77 ± 0.03
C22:4n-6	0.21 ± 0.01
C22:5n-6	0.07 ± 0.005
Total n-6 PUFA	16.64
n-3 PUFA	
C18:3n-3	1.36 ± 0.005
C20:4n-3	0.13 ± 0.01
C20:5n-3	0.09 ± 0.01
C22:5n-3	0.21 ± 0.01
C22:6n-3	0.45 ± 0.03
Total n-3 PUFA	2.24
Total PUFA	18.88 ± 0.36
Total n-6/ total n-3	7.42
AA/EPA	8.55
AA/LA	0.05
DHA/ALA	0.33

Table 1: Fatty Acid Composition of Breast Milk

Total lipids were extracted from breast milk samples of women from NL as explained under the methods section. Values are presented as mean ± SEM, n=47. Where, SFA= saturated fatty acid, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acid, AA= arachidonic acid, EPA= eicosapentaenoic acid, LA=linoleic acid, DHA=docosahexaenoic acid.

Total n-6 to n-3 PUFA and AA to EPA ratio of breast milk and cord blood: The mean ratio of n-6 to n-3 in both breast milk (Table 1) and cord blood (Table 2) was found to be 7.42 and 5.01 respectively. The mean AA to EPA ratio in breast milk (Table 1) and cord blood (Table 2) samples was 8.55 and 53.23 respectively.

Fatty Acids (% w/w)	Cord Blood
Saturated Fatty acids	
C14:0	0.66 ± 0.02
C15:0	0.16 ± 0.01
C16:0	25.86 ± 0.18
C18:0	13.02 ± 0.28
C20:0	0.35 ± 0.01
C22:0	0.63 ± 0.03
C24:0	1.50 ± 0.07
Total SFA	42.18
Monounsaturated Fatty acids	
C14:1	0.03 ± 0.002
C16:1n-9	0.48 ± 0.02
C16:1n-7	2.18 ± 0.08
C16:1n-5	0.06 ± 0.003
C18:1n-9	14.53 ± 0.24
C18:1n-7	2.47 ± 0.04
C24:1	1.36 ± 0.05
Total MUFA	21.11
Polyunsaturated Fatty acids	
n-6 PUFA	
C18:2n-6	7.56 ± 0.24
C18:3n-6	0.2 ± 0.01
C20:2n-6	0.25 ± 0.02
C20:3n-6	2.89 ± 0.08
C20:4n-6	13.84 ± 0.24
C22:4n-6	2.10 ± 0.06
C22:5n-6	0.82 ± 0.03
Total n-6 PUFA	27.66
n-3 PUFA	
C18:3n-3	0.11 ± 0.01
C20:4n-3	0.02 ± 0.002
C20:5n-3	0.26 ± 0.02
C22:5n-3	0.49 ± 0.02
C22:6n-3	4.64 ± 0.16
Total n-3 PUFA	5.52
Total PUFA	33.18 ± 0.41
Total n-6/ total n-3	5.01
AA/EPA	53.23
AA/LA	1.83
DHA/ALA	42.18

Table 2: Fatty Acid Composition of Cord Blood

Total lipids were extracted from cord blood samples of women from NL as explained under the methods section. Values are presented as mean ± SEM, n=47. Where, SFA= saturated fatty acid, MUFA= monounsaturated fatty acids, PUFA= polyunsaturated fatty acid, AA= arachidonic acid, EPA= eicosapentaenoic acid, LA=linoleic acid, DHA=docosahexaenoic acid.

Delta-6 desaturase has higher preference for n-3 PUFA in breast milk, whereas the preference is higher for n-6 PUFA in cord blood: Delta-6 desaturase activity was calculated for breast milk and cord blood samples based on the ratio of 20:4n3 to 18:3 n3 for n-3 PUFA, and 20: 3n6 to 18: 2n6 for n-6 PUFA. For breast milk, the ratio of 20:4n3 to 18:3n3 and 20:3n 6 to 18:2n 6 was 0.095 and 0.045 (Figure 4A), while the ratio for cord blood was 0.18 and 0.38, respectively (Figure 4B).

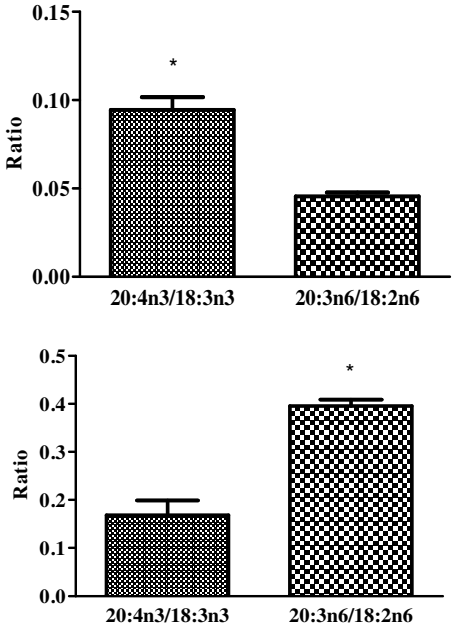

Figure 4: Delta-6 desaturase activity for omega (n)-3 and n-6 polyunsaturated fatty acids in breast milk (A) and cord blood (B) of women from Newfoundland and Labrador. Delta-6desaturase activity was calculated as explained under the methods section (n=47). Each Bar represent ratio of specific fatty acids, mean ± SEM (n=47). * indicated significantly difference of $p<0.0001$

Discussion: Breast milk and cord blood are the only source of n-3 and n-6 PUFA for prenatal and postnatal development of a child who is being exclusively breastfed. The aim of the present study was to measure the levels of n-3 and n-6 PUFA in breast milk and corresponding cord blood samples of women from NL, and to establish whether the levels of these fatty acids are comparable to the previously reported data from Canada. Our findings have shown for the first time that breast milk of women from NL have higher levels of AA and DHA compared to the previous reports from Canada [24,25].

The total amount of saturated and monounsaturated fatty acids in breast milk from women of NL was similar to the previous report from Vancouver [24,25]. Although we are able to show a direct comparison of our data to the study from Vancouver because of similar time and method of breast milk collection (within two weeks after delivery), the study from Canada compared nine countries and the time of breast milk collection was from 1-12 months. The average levels of AA in breast milk of women from NL was 0.77% ± 0.03 (n=47), whereas AA levels previously reported from Vancouver was 0.5% ± 0.03, (n=17) [24] and for Canada was 0.37% ± 0.01, (n=48) [25]. The average breast milk DHA levels of women from NL was 0.45% ± 0.03 (n=47), whereas the reported value for Vancouver was 0.22% ± 0.02 (n=17) [24] and for Canada was 0.17% ± 0.01, (n=48) [25]. Our findings show that breast milk of women from NL have significantly higher levels of AA and DHA compared to other reports from Canada and Vancouver [24,25]. Interestingly, the World Health Organization (WHO) and the Food and Agricultural Organization of the United Nations recommended levels for DHA and AA supplementation in term infant formulas are similar to our findings (DHA= 0.38%, AA= 0.7%) [26-29]. Thus, the levels of DHA and AA of breast milk from women of NL, though higher than the reported values from Canada, are comparable to those recommended by WHO [27-29].

Studies have shown that the amount of long chain n-3 and n-6 PUFA in breast milk is a reflection of dietary intake of these fatty acids [30]. Moreover, the conversion rate for LA to AA is generally low, and 90% of AA in breast milk is either from diet or from the stored body fat [31]. The mean AA to LA ratio in our study was 0.05, suggesting that the source of AA is not from its precursor LA but is likely due to a higher dietary consumption of AA or from stored body fat. We compared our breast milk fatty acid data with the previously publish study from Vancouver [24] and Canada [25], since our breast milk collection method and time was similar to these studies. The level of total n-6 PUFA in breast milk of women from NL was higher (16.64%) compared to reports from Vancouver (14.8%) and Canada (12.57%). Furthermore, we also found higher levels of LA (14.30%) in NL compared to Vancouver (13.4%) and the rest of Canada (11.48%) [24,25]. Studies using rodents have shown that high levels of LA promotes weight gain [32]; whether higher levels of LA found in breast milk of women from NL is a predisposing factor to a higher rate of obesity in this province needs to be investigated in the future.

Linoleic acid and alpha-linolenic acid (ALA), the two essential fatty acids, are metabolized to longer chain PUFA such as AA, EPA and DHA via desaturation and elongation process [33]. Delta-6 desaturase is the rate limiting enzyme in the desaturation process and this is an important step to drive the endogenous synthesis of longer chain PUFA [34]. Delta-6 desaturase is highly expressed in human mammary gland and plays an important role in breast milk fatty acid composition [35]. It was interesting to note that although the breast milk of women from NL contained higher level of LA compared to ALA (14.30% v/s 1.36%), the preference of delta-6desaturase was two times higher for n-3 PUFA compared to n-6 PUFA ($P<0.0001$). This is possibly due to a higher demand for converting ALA to DHA during lactation for the proper growth and development of the newborn [36].

Umbilical cord blood is the only source of nutrients for growing fetus. Several studies have established an association between n-3 and n-6 PUFA levels in cord blood with the risk of atopic disease [37], developmental issues such as vision, immunity [38] and neuronal development in the child [39]. A large national cohort study (Nurses' Health Study II (NHS II) has recently shown that the quality of maternal dietary fat intake, especially a diet low in n-3 PUFA during pregnancy, is associated with autism spectrum disorders in the child [40]. Requirement of n-3 PUFA in pregnancy is especially important during the last trimester because 70% of brain neuronal cells are developing at this time [39]. An increase in DHA and AA levels in the cord blood serum is associated with better performance on behavioral screen of the child, such as hyperactivity and emotional symptoms [41]. The levels of cord blood LA and ALA were lower (7.56 and 0.11%, respectively), compared to the levels of AA and DHA (13.84 and 4.64%, respectively); this is contradictory to the observations for breast milk. The cord blood samples were collected immediately after delivery, where higher levels of AA may have been required for prostaglandins synthesis at the time of child birth for uterine contractions [42]. Furthermore, the calculated activity of cord blood delta-6 desaturase indicates greater preference for n-6 pathway over the n-3 PUFA pathway ($P<0.0001$).

Ratios of n-6 to n-3 fatty acids are well known to correlate with major chronic diseases such as obesity, cardiovascular complications, cancer and developmental issues in children [43]. Studies have shown a strong correlation between the ratios of individual and total n-6 and n-3 PUFA in breast milk and cord blood with the risk of immunity, allergic manifestations and cardiovascular disease [41, 44-46]. Furthermore, the breast milk AA to EPA ratio has been shown to be associated with allergic manifestations in the new born [46,47]. In our study, the mean ratio of AA to EPA in breast milk was 8.55, while the mean ratio of total n-6 to n-3 PUFA was 7.42; cord blood mean AA to EPA ratio was 53.23, and total n-6 to n-3 PUFA ratio was 5.01. Duchen *et al*., has previously shown that a ratio of 8.7 for AA to EPA, and a ratio of 6.4 for total n-6 to n-3 PUFA in breast milk was associated with allergic developmental symptoms at the age of 18 months [45]. Furthermore, these authors also reported that a ratio of 53.4 for AA to EPA in the cord blood was associated with atopy in children. We observed a similar ratio of 53.23 for AA to EPA in cord blood. This could explain why children born to the mothers in NL may be at a higher risk of atopy. In another study by Johansson *et al*., a lower breast milk EPA (0.10 ± 0.1) level and a higher total long chain n-6 PUFA to total long chain n-3 PUFA ratio (1.8 ± 0.09) ($n=16$) was associated extensively with allergic diseases including eczema in mother [47]. The average EPA level in our study was 0.09 % and total long chain n-6 PUFA to total long chain n-3 PUFA ratio was 7.42 in breast milk. This could explain the high incidence of food allergies in children from the NL population, perhaps due to high total n-6 PUFA in the breast milk and cord blood of women living in the province of NL. Further studies will focus on measuring allergic manifestations in these children.

In conclusion, this is the first study to report that the breast milk and cord blood samples of women from NL contain higher levels of AA and DHA compared to other Canadian data. The values for AA and DHA in breast milk of women from NL are compatible with those reported by WHO for supplementation in infant formula. However, our study shows that women in NL have high levels of LA in breast milk samples. Furthermore, there was a higher ratio of AA to EPA and total n-6 to n-3 PUFA in breast milk and cord blood confirming a higher amount of total n-6 PUFA. Higher amount of LA in breast milk, as well as cord blood, suggests high dietary intake of LA; thus future research should focus on dietary intake of pregnant women from NL to establish a relationship of breast milk and cord blood fatty acid composition with dietary intake. Furthermore, NL used to be a fishing community, with high intake of seafood and marine products enriched in n-3 PUFA. Thus, future studies should focus on the current dietary habits of pregnant women living in NL and the impact on breast milk fatty acid composition.

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