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Nervonic acid is much lower in donor milk than in milk from mothers delivering premature infants—Of neglected importance?

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ABSTRACT

Nervonic acid is important for white matter development and its incorporation increases rapidly in the last trimester, but few studies focus on this fatty acid. Other mother's milk, usually after term delivery, is often used for premature infants, whose mothers cannot breastfeed. The fatty acid (FA) concentrations were analyzed by gas chromatography in 12 samples of donor human milk (DHM) from five mothers, and compared to milk from 42 mothers delivering preterm infants. Fat, lactose and protein contents were compared.

Nervonic acid showed sevenfold higher concentrations and LCPUFA 90% higher concentrations in premature milk compared to DHM. Linoleic acid was found in 43% higher concentrations in DHM than in premature milk. The fat and protein contents were lower in DHM. Our results suggest that studies are warranted to investigate if DHM given to premature infants may require supplementation of nervonic acid, and not only LCPUFA, protein and minerals.

1. Introduction

Human milk is the normative for infant feeding and has properties that cannot be compensated for in formula [1], and therefore it has been a custom in many countries to feed the vulnerable premature infant with donor human milk (DHM), when the mother's own milk is lacking or insufficient. DHM is usually received from mothers delivering at term and who during the lactation period have an affluence of breast milk. It is well known that term milk has lower concentrations of minerals, protein and long-chain polyunsaturated fatty acids (LCPUFA) than milk from mothers delivering prematurely, but fortification of DHM is only providing protein and minerals [2]. In many countries, like Sweden, DHM is often provided after feeding the mother's own baby, which might give higher amounts of fat, since hind milk is considered to have higher fat concentrations than fore milk [3].

A previous study has shown that the correlation of verbal and performance intelligence test scores at 15 years of age to early breast milk feeding were even stronger correlated after exclusion of infants provided with banked milk [4]. In the same study total brain volume was significantly related to the percentage of own mother's milk provided to the infants, as was the white matter volume for both absolute and relative brain volumes. Myelinisation is a key factor for brain volume increase [5]. Nervonic acid (NA, 24:1ω9) is important for the development of the nervous system and early myelinisation, and impaired white matter can result in cerebral palsy [6]. The supplementation of human milk with docosahexaenoic acid (DHA 22:6ω3) and arachidonic acid (AA, 20:4ω6) has been considered to contribute to a better psychomotor and cognitive development [7]. However, Cochrane reports have not given conclusive results regarding the beneficial effect of LCPUFA supplementation to preterm or term infants [8,9]. This may be a matter of dosage [10], but a possible significance of other FA, like NA, has not been given attention.

The aim of this study was to underline the difference between DHM and milk of prematurely delivering mothers (PM) regarding NA and LCPUFA, in order to focus on preterm infant nutrition in a broader perspective.

2. Material and methods

2.1. Breast milk after preterm delivery.

A collection of 42 milk samples from 42 mothers, who had delivered preterm infants after an average (SD) of 33 (2.6) weeks of gestation, was analyzed for FA pattern, and most results have previously been reported [11]. The breast milk samples were collected over 24 h, on average one week after delivery using an electric breast pump. An aliquot of the 24 h sample was frozen at −70° until analyzed as previously reported [11]. The mean (SD) age of the mothers was 29.8 (6.0) years with BMI 24.9 (3.9) kg/m^2.

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Only five mothers (13%) were obese. Detailed data of the mothers have previously been reported [11].

2.2. Breast milk after term delivery, banked milk.

A total of 12 milk samples were collected between the 6th and the 99th day after delivery from five mothers donating to the milk bank of the Neonatal Ward of Borås Children's Hospital. All donor mothers were healthy, non-medicating, non-smokers, non-substance abusers and tested negative for HIV, human T-lymphotrophic virus, hepatitis B virus, hepatitis C virus, malaria and active tuberculosis. No vegans were approved as donors. The milk samples were collected after their full term infants had been fully fed and milk was obtained by an electric pump from both breasts after each feeding. Milk was analyzed before the samples were pasteurized and kept at −70° until analysis.

2.3. Fatty acid analysis of breast milk

After lipid extraction and derivatization as methyl esters the FA in breast milk were identified and quantified on gas chromatography as previously described [11].

2.4. Macronutrient analysis of breast milk

The 12 donor milk samples were compared to milk samples from mothers of 11 premature infants for fat, protein and lactose (g%) (Milkoscan Minor 6, FOSS, Denmark).

2.5. Ethics

The study was approved by the Ethics committee of the University of Gothenburg. Informed consent was obtained from all mothers.

2.6. Statistical analysis

A mean was calculated for the results of milk sample analyses from each mother donor and the five means were compared to the 42 samples of mothers of the premature infants. Analyses were performed using SPSS 17.0 (SPSS Inc. Chicago IL USA). Independent samples' *t*-test was used to calculate the statistical difference between the two groups. Levene's test was used for correction when indicated. Mean and standard deviation (SD) is given if not otherwise indicated. The level of significance was defined at $p < 0.05$.

3. Results

The expected lower concentrations of LCPUFA in DHM were confirmed, as were the higher concentrations of linoleic (18:2ω6 LA) and α-linolenic (18:3ω3 ALA) acids (Table 1). PM had twice the concentration of DHA compared to DHM (p=0.001), and the concentration of NA was seven times higher (p=0.006) (Table 1). The NA concentration in PM was not related to gestational age. The LCPUFA, AA, and DHA, did not differ between milk samples from individual donor mothers in relation to length of lactation, but between mothers (Fig. 1). The already low concentration of NA in DHM decreased even further during time of lactation (Fig. 1).

The total ω3 FA was found in similar concentrations in DHM and PM. The much higher total ω6 FA concentration in DHM was due to high concentration of LA, while both di-homo-gamma-linolenic acid (20:3ω6, DHGLA) and AA concentrations were much lower in DHM compared to PM. The mean ratio of ω6/ω3 FA was thus much higher in DHM.

Table 1
The concentrations of major fatty acids (mol%) in donor human milk (DHM) and in milk from mothers to premature infants (PM). The mean+SD of DHM is based on mean from multiple samples of each donor mother (n=5). Independent samples' *t*-test.

Fatty acid	DHM n=5	PM n=42	p value
C12:0	4.08 ± 0.82	4.76 ± 2.43	0.535
C14:0	5.90 ± 0.94	8.09 ± 2.92	0.105
C16:0	25.32 ± 1.49	27.27 ± 2.05	0.046
C18:0	7.32 ± 0.76	6.94 ± 1.19	0.484
Total SFA	43.34 ± 1.49	48.09 ± 5.47	< 0.0001
C14:1ω5	0.20 ± 0.02	0.23 ± 0.06	0.250
C16:1ω7	2.44 ± 0.35	2.69 ± 0.6	0.367
C18:1ω9	37.92 ± 2.36	35.98 ± 4.0	−0.295
C24:1ω9 (NA)	0.05 ± 0.01	0.35 ± 0.23	0.006
Total MUFA	40.61 ± 2.57	39.25 ± 4.12	0.477
C16:1 ω 7T	0.03 ± 0.01	0.06 ± 0.02	0.011
C18:1 ω 9T	0.12 ± 0.02	0.16 ± 0.1	0.016
Total trans FA	0.16 ± 0.03	0.23 ± 0.1	0.002
C 20:3w9 (mead)	0.02 ± 0.01	0.04 ± 0.01	0.007
Ratio 20:3w9/20:4w6	0.10 ± 0.027	0.08 ± 0.027	0.250
C18:2ω6 (LA)	12.96 ± 2.25	9.06 ± 1.85	< 0.0001
C18:3ω6	0.08 ± 0.02	0.05 ± 0.04	0.155
C20:2ω6	0.35 ± 0.08	0.35 ± 0.08	0.445
C20:3ω6	0.23 ± 0.02	0.42 ± 0.12	< 0.0001
C20:4ω6 (AA)	0.30 ± 0.09	0.53 ± 0.10	< 0.0001
C22:2ω6	0.02 ± 0.00	0.06 ± 0.02	< 0.0001
Total ω6 FA	13.94 ± 2.23	10.47 ± 1.93	0.001
C18:3ω3 (ALA)	1.65 ± 0.28	1.30 ± 0.40	0.061
C20:3ω3	0.04 ± 0.01	0.09 ± 0.03	< 0.0001
C20:5ω3 (EPA)	0.06 ± 0.02	0.09 ± 0.03	0.095
C22:6ω3 (DHA)	0.19 ± 0.09	0.44 ± 0.15	0.001
Total ω3 FA	1.94 ± 0.30	1.91 ± 0.46	0.909
Ratio ω6/ω3	7.22 ± 0.53	5.60 ± 0.91	< 0.0001

As expected Mead acid (20:3ω9, eicosatrienoic acid) concentration was significantly lower in DHM (0.02 mol%) than in premature milk (0.04 mol%), as were the total saturated FA (SFA) concentrations ($p < 0.0001$).

The mean (SD) contents of fat and protein were lower in DHM than in PM, but the lactose content did not differ (Table 2).

4. Discussion

The major finding in our study was the much lower concentrations of NA in DHM than in PM. This finding might be of importance since several studies have shown the importance of NA for neurological development [4,12]. The lower LCPUFA concentrations in DHM, i.e. term milk, compared to PM corroborated results of many earlier studies. Despite that the DHM was expected to be hind milk and to have the highest fat content [3], it was lower than in PM, indicating that the supply of LCPUFA to the infants would not be compensated for. This low fat content in DHM has previously been observed [13], indicating that the low fat content can contribute to lower energy supply than expected. The high LA and ALA concentrations could not compensate for lower LCPUFA concentration, since the transformation to LCPUFA is not efficient in the newborns [14]. Also the supplements of LCPUFA in recent formula to preterm infants might not be sufficient as reflected in the decrease of both DHA and EPA in plasma phospholipid FA analyzed in preterm infants, who had to start formula feeding before 44 weeks of gestational age compared to those who continued exclusive breastfeeding [11]. Furthermore follow up examinations in preterm infants indicate that high LA concentration in the neonatal period might have negative influence on the development up to 18 months of corrected age [15,16].