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Article

Impact of maternal nutrition and perinatal factors on breast milk composition after premature delivery

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Abstract: (1) Background: Premature infants require mothers' milk fortification to meet nutrition needs, but breast milk composition may be variable leading to a risk of inadequate nutrition. We aimed at determining factors influencing mothers' milk macronutrients. **(2) Methods**: Milk samples were analyzed for the first 5 weeks after premature delivery, by infrared spectroscopy. Mothers' nutritional intake data were obtained during standardized interviews with dieticians then analyzed with reference software. **(3) Results**: Composition of 367 milk samples from 81 mothers was (Median [range]g/100mL): Carbohydrates 6.8[4.4-7.3], lipids 3.4[1.3-6.4], proteins 1.3[0.1-3.1]. There was a relationship of milk composition with mothers' carbohydrates intake only (r=0.164; p<.01). Postnatal age was correlated with milk proteins (r=-0.505 p<.001) & carbohydrates (r=+0.202, p<.001). Multiple linear regression analyses showed (coefficient) a relationship between milk proteins r=0.547 and postnatal age (-0.028), carbohydrates intake (+0.449) and the absence of maturation (-0.066); and between milk lipids r=0.295 and carbohydrates intake (+1.279) and smoking (-0.557). Finally, between milk carbohydrates concentration r=0.266 and postnatal age (+0.012) and smoking (-0.167). **(4) Conclusions**: Variability of mothers' milk composition is differentially associated for each macronutrient with maternal carbohydrates intake, antenatal steroids, smoking, and postnatal age. Improvement in milk composition could be achieved by modification of these related factors.

Keywords: Maternal nutrition; Breast milk; Premature delivery; Milk composition

1. Introduction

Premature infants require mothers' milk fortification to meet their nutrition needs [1]. This fortification is usually standardized using an assumed macronutrient milk composition [2]. However, studies suggest that breast milk composition variability may be much wider than expected [3,4], leading to inadequate newborn nutrition. McLeod et al. performed a survey of protein and energy intakes by milk analysis within the first 28 days of life in 63 infants born before 33 weeks gestation to assess their effect on growth [3]. Their results show that breast milk composition vary for all macronutrients with median protein concentrations

of 16.6 g/L ranging from 13.4 to 27.6 g/L, and median caloric intake of 73.3 Kcal/100mL ranging from 63 to 93 Kcal/100mL. Of note, actual protein intake was correlated with infants' growth. The authors conclude that preterm milk composition is very variable and routine fortification using assumed averaged composition may result in inadequate nutrition with slower weight gain as observed in their study [3].

Inadequate nutrition could indeed explain in part the postnatal growth restriction observed in many premature infants [5,6]. We aimed at determining factors, including mothers' nutritional intake, which may be associated and explain breast milk macronutrient variability after premature delivery before 34 weeks gestational age.

2. Materials and Methods

This is an observational study using milk bank data. In our level III Maternity Hospital, mothers' own milk is pasteurized for premature infants. After pasteurization, we routinely analyze breast milk composition to verify the appropriateness of standardized fortification. For the purpose of the study we collected data for all milk batches each mother provided to the milk bank regardless of the time of the milk collection to evaluate milk composition variation throughout the first 5 weeks of lactation. Each batch is a pool of 1-3 days depending of the volume collected and frozen by each mother at home. Once a volume of at least 500 mL was collected, the mother would hand over the frozen batch to the milk bank for pasteurization. Hand-over occurred daily to twice a week.

Mothers who delivered a premature infant before 34 weeks gestation at our unit were enrolled within 5 days after delivery when they announced their choice for breastfeeding. Mothers' dietary preference and nutritional intake data were obtained during personalized interviews with experienced dieticians. They used standardized questionnaires based on validated documents in the National French survey. The dieticians recalled dietary intake information from the 2 weeks prior to the interview. Perinatal data were prospectively collected at the time of the interview and from the mothers' file. We analyzed maternal diet and macronutrient intake from the recall data averaged per day with an appropriate software (Geni® V7.0, Micro6, Villers les Nancy-F).

For milk analysis, we used the same protocol as described in the literature [7,8] and advised by the manufacturer. In short, mothers milk pools over 1-3 days are delivered frozen (-20°C) to the milk bank, thawed, pasteurized, aliquoted in bottles of 50 ml, and stored at -80°C. Two samples of 1 ml from 2 different bottles of each batch were analyzed after homogenization by ultrasound to compensate for milk thawing and to verify the quality of the homogenization. We used infrared spectroscopy pre-calibrated against a chemical reference with analytical accuracy <0.1g/100mL (Miris AB® V3, Uppsala, Sweden). Because it has been shown that mid-infrared analyzers may require calibration adjustment, we verified the calibration daily to ensure appropriate measures [9]. The samples were rejected if variability was over 10%, otherwise the average of the 2 values was kept for further analysis.

We started our statistical analyses by power calculation relying upon McLeod [3]: We calculated that to demonstrate a significant relationship with a coefficient of at least r=0.550, considered clinically significant, with an alpha risk of 0.016 (Bonferroni correction for the 3 nutrients) and a Power of 0.90, 78 mothers with at least 2 samples would be needed (Power and Precision™ V4, Biostat Inc, Englewood, NJ, USA 2001). Normally distributed data, assessed by a Shapiro-Wilk test of normality, are presented as mean values with standard

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deviation (SD), the median and the interquartile range (IQR); non-normally distributed data are presented as medians with IQR only. To evaluate differences between groups, we used the Student t test for continuous variables and the Chi2 test or Fisher exact test when appropriate for categorical variables. For continuous variables not normally distributed, we used the Mann-Whitney U test. To determine which variables were associated with milk composition, we performed a bivariate analysis then a stepwise multiple linear regression, including all variables associated with milk composition in bivariate analysis, with a tolerance set at 10^{-5} the probability to remove set at 0.15 and a Confidence Interval at 0.95. Observed differences were considered statistically significant if $P<0.05$. Statistical analysis was performed with SYSTAT 12 software (2007, Systat Software Inc. San Jose CA, USA).

The study has been approved by our Institutional Ethics and Review Board on March, 9th 2013 (Number: MRU13-02).

3. Results

3.1 Description of the studied population:

From August 2013 to January 2014, 367 milk samples were obtained from 81 mothers (Figure 1). Two to 10 batches were collected from the mothers (Median (IQR): 3 (2-6)).

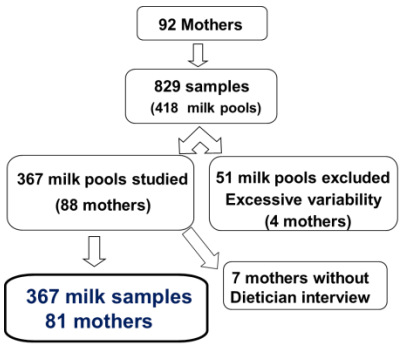

Figure 1: flow chart.

The mothers involved in the study were 29 years old [19-42] (median [range]). Their height was 1.64 m [1.53-1.85] for a weight of 63 Kg [42-110] and a body mass index (BMI) of 23.2 [16.4-43]. Weight gain during pregnancy ranged from 0 to 30 Kg (mean 10.2 Kg). Age of delivery was 31 weeks gestational age [24-34]. Twenty (25%) mothers smoked during pregnancy; 63 (78%) were single pregnancies and 19 (23%) presented with toxemia. Prenatal maturation with corticosteroids was achieved in 37 (46%) mothers and partial maturation in 33 (41%). Vaginal delivery occurred in 42 mothers (52%) and cesarean section in 39 (48%).

Forty-nine (60%) newborns were males; neonatal adaptation was good with an Apgar score above 6 at 1 and 5 min for all infants. Their mean birth weight was 1523±512 g (Median[range]=1460 [600-2500] g).

3.2 Maternal nutritional intake and milk composition:

Maternal nutritional intake was 2169±562 Kcal/d (2146 [1197-3628]) with 88±28 g/d (88[40-213]) of fat intake, 86±20 g/d (87[40-160]) of protein intake and 257±81 g/d (247 [103-533]) of overall carbohydrate intake.

The global milk sample composition was (Median [range]/100mL): carbohydrates 6.8g [4.4-7.3], lipids 3.4g [1.3-6.4], proteins 1.3g [0.1-3.1]. The correlation between mothers' food intake and milk composition is shown in Table 1.

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Table 1: linear regression between mothers' food intake and milk composition (coefficient r):

Nutrients intake	Milk Calories	Milk Proteins	Milk Lipids	Milk Carbohydrates
Log Energy	0.110*	0.094	0.106*	0.034
Protein	0.01	0.03	0.01	0.04
Fat	0.03	0.04	0.03	0.03
Carbohydrates	0.131**	0.109*	0.127*	0.035

(* p<0.05. ** P<0.01)

3.3 Perinatal factors effect on milk composition:

3.3.1. Postnatal age effect on milk composition:

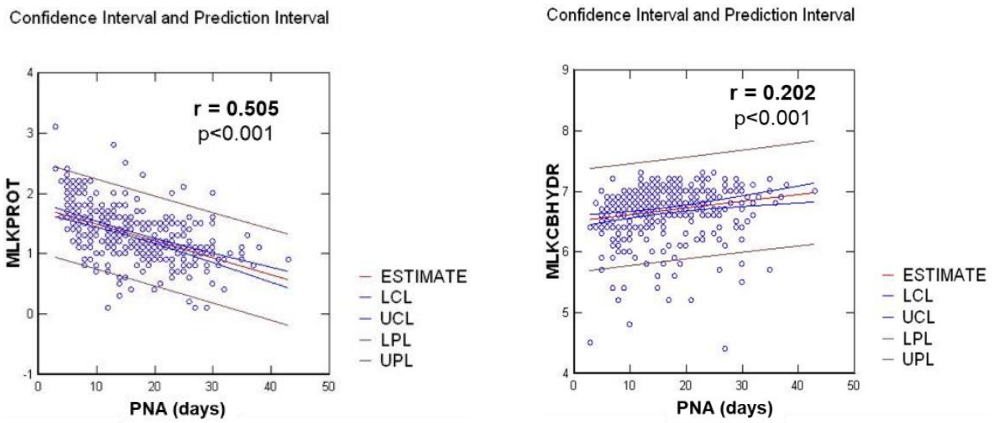
Weekly mean protein content significantly decreased for the first 4 weeks post-delivery then remained stable on week 5. We observed a comparable but inverse evolution for carbohydrates and there was no significant difference over the first 5 weeks after delivery for fat content of mothers' milk (Table 2).

Table 2: Average milk composition per postnatal week

week	1 (n=52)	2 (n=125)	3 (n=91)	4 (n=68)	5 (n=31)
protein	1.78±0.39*	1.40±0.40*	1.26±0.34*	1.08±0.36*	1.05±0.40
lipids	3.23±0.80	3.58±0.98	3.59±0.97	3.41±0.96	3.40±1.06
CHO	6.50±0.43*	6.66±0.38*	6.70±0.46*	6.81±0.44*	6.75±0.44

*<0.01

We observed a significant linear regression for carbohydrate and an inverse correlation for protein contents shown in Figure 2.



(**a**) (**b**)

Figure 2. Linear regression between milk composition in g/100mL and postnatal age (PNA) in days, for: (**a**) Protein milk content (MLKPROT); (**b**) Carbohydrate milk content (MLKCBHYD)

3.3.2. Perinatal factors impact on milk composition in bivariate analysis:

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There was no relationship with milk composition for mothers' age, weight, height or BMI before pregnancy. There was no relationship either for the mode of delivery, multiple pregnancies, toxemia or gestational age at delivery. We observed a weak association between weight gain during pregnancy and milk lipid content (r=0.117, p=0.026). Finally, we observed a significant correlation between milk composition and antenatal steroid maturation, smoking during pregnancy, and an inverse relationship with the infants' birth weight. Detailed data are shown in Table 3.

Table 3: confounding perinatal factors for milk composition:

Milk content (Mean, g/100ml)	**Smoking**		**Antenatal steroids**		**Birth weight** (linear regression: r)
	Yes	**No**	**Yes**	**No**	
Lipids	3.10	3.59*	3.6*	3.1	0.082
Carbohydrates	6.57	6.71*	6.7	6.6	- 0.259*
Protein	1.34	1.33	1.3	1.2	- 0.318*
Calories	62.8	67.8*	67.6*	61.9	- 0.106

(* $p<0.05$)

3.3.2. Stepwise multivariate regression analysis of factors associated with milk composition in bivariate analysis:

All factors showing a significant association with mothers' milk content in bivariate analysis were included in the model presented in Table 4.

Table 4: Multivariate analysis of factors associated with mothers' milk content:

Milk content	**Postnatal Age**	**Carbohydrate intake**	**Smoking**	**No steroids**	**Weight gain**
Lipids (r^2=0.087)	NS	1.279*	- 0.557*	NS	NS
Carbohydrates (r^2=0.071)	0.012*	NS	- 0.167*	NS	NS
Protein (r^2=0.299)	- 0.028*	0.449*	NS	- 0.066*	NS
Calories (r^2=0.101)	NS	14.053*	- 5.901*	NS	NS

(* $p<0.05$)

4. Discussion

Our study confirms that breast milk composition in macronutrients has a large variability as suggested in previous studies [3,4]. Therefore, fortification on assumed averaged macronutrient in milk leads to inadequate nutritional intake for most premature infants. Our data show that indeed maternal nutrition may influence breast milk macronutrient composition in mothers who delivered prematurely. Our data also show that overall carbohydrate only is positively correlated with protein, fat and caloric density. To our knowledge, this observation has not been yet reported. In their study comparing breast milk nitrogen content of mothers from 2 different Chinese areas, Zhao et al found no significant difference in 18 studied amino-acids despite significant lower protein intake in

one of the studied areas [10]. This is consistent with our results. Likewise, in their study on maternal supplementation with omega-3 precursors, Mazurier et al. showed a qualitative alteration with increased alpha-linoleic acid content but no significant modification of the overall lipid concentration [11]. In a recent survey on food and nutrient intake of women in France, Hebel et al. showed that few lactating mothers met the nutritional guidelines and may therefore be at risk of food and nutrient inadequacies [12]. One could speculate that improving the maternal nutritional balance, especially an appropriate overall carbohydrate intake, might contribute to improved milk composition for preterm infant feeding.

Longitudinal analysis of milk composition showed a significant decrease in milk protein content with an inverse correlation for carbohydrate and a stable lipid concentration over the first 4 weeks after delivery. These data are consistent with the publication of Maly et al. [8] who found the same evolution for the 3 macronutrients and Mahakan et al [4] who found a 50% decrease in protein concentrations with a 30% increase in carbohydrate concentrations over the first 28 days after delivery. From his review analysis on human milk in premature infants [13], including Charpak et al [14] and Bauer et al studies [15], Underwood evaluated the changes in milk composition over 8 weeks from delivery and found the same results as in our study over this longer assessment period. Thus, this evolution is important to take into account when considering fortification of human milk for premature infants.

Few data are available for the impact of perinatal factors on milk composition. In our study, we did not find any significant association with mothers' age, weight, height or BMI before pregnancy. In their study on maternal nutrition and body composition during breast feeding, Bzikowska-Jura et al [7] found a variance in milk fat content related to BMI. However, they studied actual BMI at 3 time points when we differentiated BMI before pregnancy and weight gain during pregnancy which was indeed associated with milk fat content. Thus, our results are consistent with published data but suggest that it is not BMI per se, but rather weight gain during pregnancy that may be associated with milk composition. Also, there was no relationship for the mode of delivery, multiple pregnancies, or toxemia. As in our study, Maly et al. [8] showed no effect of the degree of prematurity at delivery on milk composition. However, we found a moderate effect of birth weight in bivariate analysis but not after adjusting for other confounding factors in multivariate analysis (data not shown). Finally, we showed that breast milk protein concentrations were positively correlated with mothers' overall carbohydrate intake and negatively correlated with duration of lactation from birth onwards and the absence of steroid maturation; breast milk carbohydrate concentrations were positively correlated with duration of lactation, and negatively correlated with smoking, as shown in Bachour et al study [16], whereas breast milk lipid concentrations were positively correlated with mothers' carbohydrate intake and negatively correlated with smoking.

Our study has strength and limitations. Strength relies upon the number of samples, the blinded collection of the data and the reproducibility of milk content measurements with a rejection when the control variability was above 0.10. Also, the dietary questionnaire over two weeks recall, given by experienced dieticians with illustrated standardized catalog for the amount of food, allows an appropriate evaluation of the averaged usual diet of the mothers. However, this questionnaire was given 2 weeks from the interview and may not strictly reflect the diet of the lactating mother at the time of milk collection. Anyhow, one may speculate that the level of macronutrients intake would be only slightly modified from the routine diet of the patients. We did not find a significant difference from the results with

sugar, fibers or overall carbohydrate therefore we presented only the results with overall carbohydrate but this would be confirmed by targeting study. Finally, because this is an observational study, we were not able to standardized the time the mothers would hand over their milk collected at home to the milk bank nor the number of collection days within each pool. However, this would only vary from one to three days and we longitudinally evaluated the results over 5 weeks. Thus, despite this study does not really present an individual longitudinal analysis, the linear regressions observed for the overall population and the multivariate analysis taking time as a confounding factor are likely to allow good reliability of our findings.

In conclusion, our study confirms that human milk macronutrients composition has a wide variability. This variability is differentially associated for each macronutrient and associated with maternal carbohydrates intake, antenatal steroids, smoking, and the delay from delivery. To improve mothers' milk composition, one could aim for improving the prenatal nutritional balance in pregnant women, particularly for carbohydrate intake, supporting an appropriate weight gain during pregnancy and advise to stop smoking. In case of expected prematurity, steroid maturation is also needed to improve milk composition. Ideally, breast milk composition would be regularly measured for individualized fortification to achieve appropriate growth of preterm infants. But when it is only possible to apply standard fortification, at least human milk composition in relation to lactation duration from birth should be taken into account. Targeting investigation should be performed and confirm the observed data.

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