The choline content of human breast milk expressed during the first few weeks of lactation.

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Choline is a conditionally essential nutrient required by the human body for synthesis of the neurotransmitter acetylcholine and the phospholipids phosphatidylcholine (PtdCho), lysophosphatidylcholine, sphingomyelin (SM) and plasmalogen. It also undergoes irreversible oxidation to form betaine, a major source of methyl groups. Betaine is excreted by human infants in large quantities (up to 1 mol/mol creatinine) during the first year reaching a maximum at 2-3 months of age. Since the betaine content of breast milk is very low and choline is the only known endogenous source of betaine, the supply of dietary choline to neonates may be critical particularly for premature babies whose intake may be restricted in the early days of life [1]. Some premature babies receive expressed breast milk and we have measured the choline content, distribution and temporal variation to determine whether its supply is sufficient. Choline is present in breast milk as free choline (Cho), phosphorylcholine (PC), glycerophosphocholine (GPC), PtdCho and SM [2]. Previous studies have not considered the contributions of GPC and PC [3].

Milk samples were obtained from the Neonatal Intensive Care Unit of the Royal London Hospital. Samples were expressed by mothers and then frozen at -20°C until such time as the baby required the milk. At that time samples were defrosted and an aliquot taken for preparation for ^{1}H nmr spectroscopy.

The water soluble metabolites (Cho, PC and GPC) were measured in perchloric acid extracts of the milk, neutralised with KOH. Samples were run at pH values of 2 and 7 to distinguish the three metabolites from each other and from other metabolites with similar chemical shift values such as carnitine. Following lyophilisation the samples were dissolved in 0.6 ml ^2H_2O with 50 µl 10mM fumarate and 20 µl 20 mM 3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionate ($TSPd_4$) for field locking, quantification and chemical shift referencing respectively. The samples were run at room temperature in a Jeol GSX500 or Bruker AM400 spectrometer using a single pulse sequence (30° pulse angle, 2.73 s acquisition time and a 5 s recycling time).

The choline containing phospholipids were measured in chloroform extracts. After separation the lower chloroform layer was evaporated under nitrogen, redissolved in 0.6 ml C^2HCl_3:$C^2H_3O^2H$ (2:1) containing tetramethylsilane (TMS; 0.03% v/v in C^2HCl_3) and 0.3 mM 1,3,5-trichlorobenzene as the chemical shift reference and quantification standard respectively. The samples were run as described above.

Figure 1 shows the concentration of choline metabolites in the milk expressed by one mother. This is qualitatively similar to the pattern observed for milk from 4 other mothers who have expressed milk over a similar time period and whose babies were born at between 28 and 37 weeks gestation. Until about one week after birth the total choline content is relatively low and is mostly present as Cho, PtdCho and SM. After that the total choline concentration rises quite markedly with the greatest increase being shown by GPC and PC which now contribute to more than half of the total choline content. Earlier studies on the choline content of human breast milk with no measurement of PC or GPC therefore gave large underestimates of dietary choline supply to neonates over a week old.

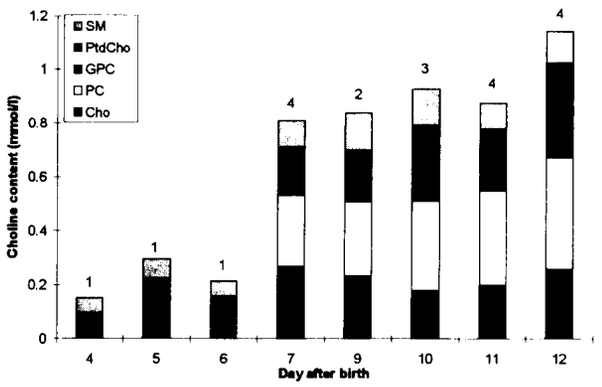

Figure 1: Concentration of choline metabolites in breast milk expressed by the mother of a 37 week gestation baby during the first two weeks after birth. The bars represent the average of each metabolite for the samples collected that day and the number above the bar is the number of samples.

Whether the low choline content of expressed breast milk during the first six days is disadvantageous is unclear. However we have found that betaine excretion during this period is considerable (27.4 ± 2.8 µmol/kg/day; mean ± sem, n=27, during the first 10 days) even in premature babies. Without an alternative supply it is possible that endogenous sources (e.g. PtdCho) may provide choline. Such an occurrence could compromise development especially of the brain where myelin formation is taking place.

This pattern of rapid increase of certain milk components during the first week of lactation has been reported in other studies [4]. As the milk volume rises between days 2 and 4 for the mother of a normal term infant there is a parallel rise in the concentration of a number of metabolites including lactose, citrate and potassium. The start of lactation itself is stimulated by birth and an accompanying fall in progesterone levels. Sustained lactation, rise in volume and increase in concentration of metabolites depends on suckling or expression. The delay in onset of milk expression for mothers whose infants require intensive care may account for the later rise in choline metabolites we have observed compared with other studies.

Acknowledgements: We thank the Wellcome Trust for support and the University of London Intercollegiate Research Service at Birkbeck College and the MRC Biomedical NMR Centre at NIMR, Mill Hill for NMR facilities. We also thank the staff of Elizabeth Ward, Royal London Hospital for assistance in the collection of breast milk samples.

Abbreviations used: Cho, choline; PC, phosphorylcholine; GPC, glycerophosphocholine; PtdCho, phosphatidylcholine; SM, sphingomyelin.

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