

^{31}P -NMR follow-up of human milk during lactation

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Many phosphorylated compounds in milk have nutritional relevance. Inorganic phosphate (Pi) is the major phosphorylated compound in milk and accounts for much of the phosphorous dietary intake. Serine phosphate (SerP) moieties in casein are related to protein content as well as to calcium transport. Choline and choline esters have an important role in the structural integrity of membranes, transmembrane signaling and normal brain development. Among choline esters, phosphocholine (PC) and glycerophosphocholine (GPC) are present in significant amounts in milk and can be easily detected by ^{31}P -NMR. The aim of this study was to analyse the phosphorylated compounds in human milk during lactation by ^{31}P -NMR.

MATERIALS AND METHODS

Breast milk was collected from three women donors that gave written informed consent. Milk was collected along 18 weeks after birth, at the same weekday and at the same time of the day, and was frozen until analysed.

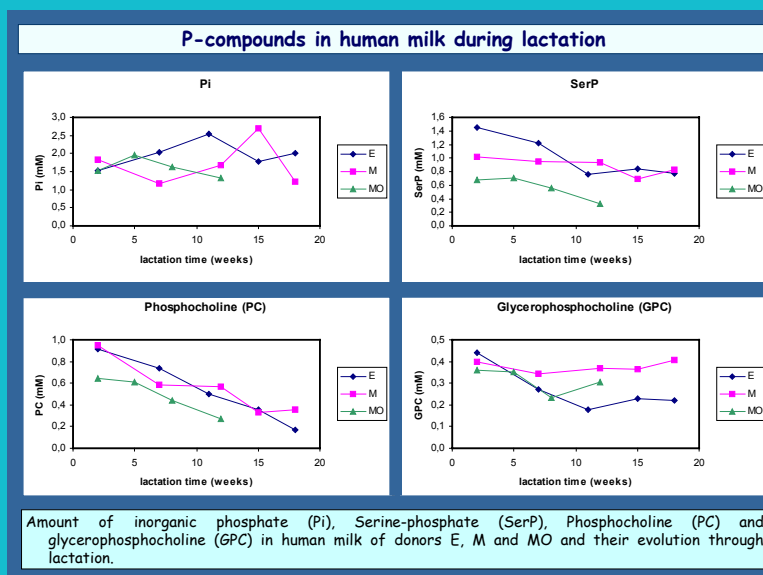
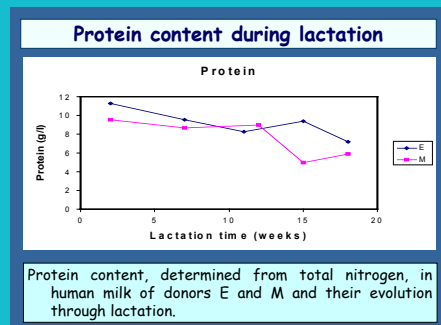
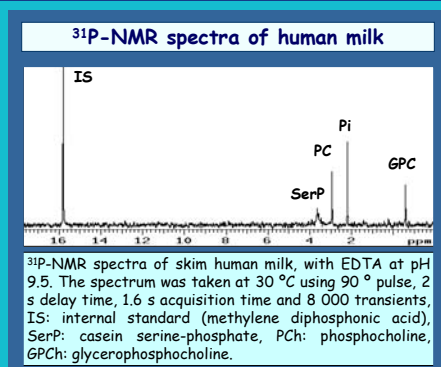
Sample preparation was as follows: 500 μL milk was mixed with 100 μL of 0.5M EDTA, 10 μL of internal standard (400 mM methylene diphosphonic acid), 300 μL D_2O and NaOD to increase the pH to 9.50. After centrifugation at 8800 $\times g$, 10 $^\circ\text{C}$ for 30 min residual fat was discarded and samples were transferred to 5 mm NMR tubes.

^{31}P -NMR spectra were obtained in a 400 MHz Varian UNITYINOVA (Varian NMR Instruments, Palo Alto, CA 94304, USA) at 30 $^\circ\text{C}$. Two type of spectra were obtained:

- 1) Quantitative spectra of the first sample of each donor, with full relaxation (90° pulse, 60 s delay time, 1.6 s detection time and 10 000 transients). These spectra were used for quantitative determination of each P-compound in the first sample and for calculation of a factor (F) able to convert results from spectra taken in (2) to quantitative values.
- 2) Spectra of all samples with partial relaxation (90° pulse, 2 s delay time, 1.6 detection time and 8 000 transients). These spectra were used to follow-up the milk from each donor, after being relaxed with factor F to quantitative results.

Protein content was determined from the total nitrogen content, analysed by the Kjeldahl method.

RESULTS



CONCLUSIONS

- 1) Values obtained were in the range of concentrations previously found by other authors
- 2) SerP (from caseins) decreased along lactation, similarly to the protein content
- 3) No clear increase or decrease was found for Pi and GPC
- 4) A clear decrease in PC was found during lactation

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