

## Identification of soluble human milk casein phosphopeptides by mass spectrometry

P. Ferranti<sup>1</sup>, M.G. Calabrese<sup>1</sup>, S. Caira<sup>2</sup>, G. Picariello<sup>2</sup>, G. Mamone<sup>2</sup>, F. Addeo<sup>1</sup>

<sup>1</sup>DSA Università Federico II, Parco Gussone 80055

<sup>2</sup>ISA-CNR, via Roma, 52 83100 Avellino

Casein is a source of biologically active peptides released following hydrolytic events which occur during gastrointestinal digestion. Antimicrobial, antihypertensive, antithrombotic, cytomodulant, immunomodulant, and opioid peptides have been detected together with mineral carrier soluble casein phosphopeptides (CPPs).

The aim of this work was to apply proteomic techniques to human milk soluble CPPs characterization. Here we describe a profiling of naturally occurring CPPs in human milk collected from four women at different time of lactation. Selective enrichment of CPPs was performed by TiO<sub>2</sub> microcolumns and subsequent identification by MALDI-TOF MS and LC-ESI-MS/MS. By means of this approach it was possible to define the proteolytic pattern from which peptides are generated in human milk and to identify the parent proteins and the specific cleavage sites. The CPPs identified in the human milk cover some phosphorylation sites of *b*-casein, *a*<sub>s1</sub>-casein, and *k*-casein. Of the different phosphopeptides found in the four milks sampled, *b*-casein and *a*<sub>s1</sub>-casein contributed both with ~90%, and *k*-casein whereas ~10%. This is in accordance with the lower abundance of *k*-casein in human milk. The most abundant primary peptides came from the N-terminal region of  $\beta$ -casein and have in common the C-terminal Lys.

This indicates the strong contribution of plasmin to the primary proteolysis of caseins. Plasmin may also be responsible for the generation of a number of CPPs from its cleavage in *a*<sub>s1</sub>-casein as the peptides bonds hydrolysed were of Lys-X type (X is any amino acid residue). The results are in agreement with other studies on the proteolysis of milk from other species and derived cheese. Our data confirm that significant proteolysis spontaneously occurs even in freshly secreted human milk by endogenous peptidase activity; this could have physiological implications as new-born gastrointestinal apparatus is not completely developed. No major differences in CPPs composition were observed among the samples as function of lactation stage, suggesting that nearly constant amount of CPPs has to be supplied with milk all along the lactation period. In newborns where the rate of formation of bone mineral is essential and the deposition of bone is regulated by several hormones, the efficiency of calcium absorption can be enhanced by CPP ingestion.