A-37: *In vitro* digestibility of proteins in formula milk by alimentry tract endopeptidases

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Hypersensitivity to cow miik proteins is frequently observed during infancy. This is largely attributed to the antigenic responses to proteins which are absorbed through the immature intestinal mucosa. Previous studies have indicated milk proteins such as β-lactoglobulin, α-lactalbumin, k casein and bovine IgG are responsible for the hypersensitivity reaction in infants. In Sri Lanka, powdered milk formulae are widely used in infant feeding. The absorption of hypersensitivity-inducing milk proteins through the intestinal mucosa will depend on the non-breakdown of these proteins by enzymes in the digestive juices. In the present study, we have examined the *in vitro* digestion of infant and whole milk formulae by pepsin and trypsin, the 2 potent endopeptidases in the alimentary tract.

Six brands of powdered milk formulae which included 3 infant milk formulae (IF 1-3) and 3 whole milk formulae (WM 1-3) were analysed. The powdered milk was reconstituted according to the manufacturer's instructions dissolving 1g of milk powder in 5ml of water. These milk samples were then subjected to proteolytic digestion by incubating 750 µl of each milk sample with 300 µl of phosphate buffer (pH 2) and 75 µl of 1% pepsin at 37°C. Another reconstituted sample was subjected to trypsin digestion by incubating the sample with pH 8 phosphate buffer and 1% trypsin. Following the initiation of the digestive process, milk samples were subjected to SDS-PAGE. 50 µl aliquots were collected at 0, 0.5, 1,. 2, 3, 4, 6. and 24h and mixed with 50 µl of SDS-PAGE sample buffer and gels were stained with 0.1% Coomassie blue R-250. Pure milk proteins (β-lactoglobulin, α-lactalbumin and casein) were used as molecular markers. All standards used were from Sigma Chemical Co., U.S.A.

Prior to digestion the milk proteins, immunoglobullins, casein, bovine serum albumin and whey proteins (β-lactoglobulin, α-lactalbumin) were clearly visible on the gels. The digestion of casein in the presence of pepsin commenced at 0.5h and it was found to be completed by 4h. In contrast, β-lactoglobulin remained as a prominent band even after 24h. Trypsin showed rapid digestion of casein that was complete by 1h but β-lactoglobulin showed only partial digestion after 24 h. Casein in infant formulae digested more rapidly than in whole milk. However, in infant formulae too, β-lactoglobulin and α-lactalbumin remained undigested even after 24h. the digestion rate of immunoglobulins and bovine serum albumin with pepsin and trypsin were similar to that of casein.

This is *vitro* study indicates that the whey proteins, β-lactoglobulin, α-lactalbumin are not readily digested by pepsin and trypsin thus allowing the possibility for their absorption through the intestinal mucosa to induce an

antigenic response that could eventually result in a hypersensitivity reaction in some individuals.

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