

## Pancreatic elastases I and II in $\beta$ -lactoglobulin hydrolysis: preliminary results

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Vitamin E:

control population:  $10.5 \pm 2.6$  mg/L all aetiologies:  $J_0$ :  $9.1 \pm 4.4$  mg/L

 $J_{365}$ : 12.1 ± 3.0 mg/L

viral aetiologies:  $J_0$ : 7.3 ± 3.5 mg/L

Trace elements:

Copper concentrations were similar to the control group whereas zinc and selenium concentrations appeared not to be significantly decreased.

The levels of vitamin A were largely decreased in all hepatocellular diseases; only 13 patients had a normal status. The levels of vitamin E were only decreased for viral pathologies. One year after liver transplantation, all the patients had a normal vitamin A and E status.

No significant difference was observed between the different pathologies.

This study shows that patients with an hepatocellular insufficiency have a deficient status in antioxidant vitamins. Therefore, systematic vitamin supplementation could be of value in order to protect the liver against free radical agression. In contrast, the necessity of trace element supplementation remains to be proven.

## **DIGESTION - ABSORPTION**

Pancreatic elastases I and II in β-lactoglobulin hydrolysis: preliminary results. C Desbois <sup>1</sup>, I Le Huërou-Luron <sup>1</sup>, M Gestin <sup>1</sup>, V Philouze-Rome <sup>1</sup>, T Lengagne <sup>2</sup>, L Roger <sup>2</sup>, F Mendy <sup>3</sup>, P Guilloteau <sup>1</sup> (<sup>1</sup> Laboratoire du jeune ruminant, Inra-Rennes, 65, rue de Saint-Brieuc; <sup>2</sup> Nutrinov, 35042 Rennes; <sup>3</sup> 1, place de Béarn, 92110 Saint-Cloud, France)

Bovine lactoserum proteins such as  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin are, together with bovine caseins, the most commonly used in infant formulas because of their high

nutritional value. These proteins differ qualitatively and/or quantitatively from those of human milk. In addition to an important casein level (80 vs 35% in human milk), cow milk contains 50% of B-lactoglobulin in lactoserum. This globular protein is missing in human milk. These cow milk components are not always tolerated; about 3% of children under 2 years of age present allergic reactions to these milk proteins. According to the literature, this intolerance reaction can be, in part, the result of a deficient proteolytic equipment in the baby's digestive tract. Because of its significant action on globular proteins, the pancreatic elastase It is probably one of the proteases implicated. This enzyme activity would be weak at birth and would increase up to 24 months of age while infant intolerance reactions decrease during this period. Moreover, elastase I, which is not expressed in the human pancreas, is present in the calf which digests its mother's milk proteins very efficiently. The aim of this study was to purify porcine elastases I and II and to analyse their role in β-lactoglobulin hydrolysis. The hydrolysis product antigenicity was measured by radial immunodiffusion.

The purification procedure of both porcine elastases from pancreas acetone powder included ammonium sulfate fractionation followed by cation-exchange chromatography on FPLC (monoS, HR5/5). All β-lactoglobulin hydrolyses were realized under the same conditions (substrate, temperature and time) with elastase I, elastase II and/or with a mixture of pepsin, trypsin and chymotrypsin used to prepare hypoallergenic milk. The reaction products were then separated by reverse-phase chromatography on high-performance liquid chromatography (HPLC) (column C18) and their antigenic properties were analysed using rabbit antisera raised against bovine β-lactoglobulin.

Analysis of HPLC chromatograms showed that elastases I and II presented

common and distinct specificities at different sites of hydrolysis. β-lactoglobulin hydrolysis with these enzymes released peptides which had lost, respectively, 26 and 72% of their antigenicity. These enzymatic specificities were complementary to those of pepsin, chymotrypsin and trypsin. The residual antigenicity of β-lactoglobulin was thus decreased 12% with the use of elastase I in addition to the gastric and pancreatic mixture. This effect was greater with elastase II which reduced the \( \beta \- lactoglobulin \) antigenicity by 35%. According to these results, both elastase enzymes, and particularly elastase II, seemed to be efficient at improving the digestion of bovine lactoserum proteins in the preparations of infant hypoallergenic formulas.

Pancreatic elastases I and II. Postnatal development in calves and pigs. M Gestin <sup>1</sup>, I Le Huërou-Luron <sup>1</sup>, C Desbois <sup>1</sup>, J Peiniau <sup>2</sup>, A Aumaitre <sup>2</sup>, R Toullec <sup>1</sup>, P Guilloteau <sup>1</sup> (<sup>1</sup> Laboratoire du jeune ruminant; <sup>2</sup> Station de recherches porcines; Inra, Rennes, France)

Globular bovine lactoserum proteins such as  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin are the most commonly used proteins in infant formulas together with bovine caseins. Intolerance to those proteins could be partially due to deficient proteolytic equipment in the baby's digestive tract. Because of its significant action on globular proteins, pancreatic elastase II is probably one of the enzymes implicated. In humans, this enzyme would be present at low levels at birth and its concentration would increase up to 24 months of age while the infant intolerance reactions are disappearing. Recent studies showed that elastase II and to a lesser extent, elastase I, had an hypoantigenic action on β-lactoglobulin. Elastase II ontogenesis has not been investigated so far in the calf (which digest cow milk proteins very efficiently) or in the pig (one of the species commonly used as a human model). The purpose of this study was to measure the postnatal development of pancreatic activities of elastases I and II in calves and pigs.

Four groups of calves (n = 18) and three groups of pigs (n = 18) were used. Calves and pigs of the first groups were sacrificed at birth while those of the remaining groups were milk-fed until slaughter on days 7, 21 and 119 for the former and on days 10 and 21 for the latter. Elastolytic activities were determined spectrophotometrically using specific synthetic substrates. Chymotrypsin and elastase I also hydrolyse the elastase II substrate, thus their activities were substracted in order to calculate the true elastase II activity.

The postnatal elastase II pattern was similar in both species: the specific activity was maximum at birth and declined sharply thereafter. Refering to the 0 day values, it was 52 and 68% lower in 21-day-old pigs and calves, respectively, and 96% lower in 119-day-old calves. In contrast, in calves the elastase I activity was five-fold greater at 119 days than at birth while in pigs it did not show any variation.

Elastases I and II (which seem to be implicated in allergenic protein hydrolysis in humans) are present as early as birth in both species. The early high level of elastase II activity would indicate an essential action in a period when the elastase I has a lower level. The change in its concentration with age is similar to that observed for other enzymes which play a prominent part in milk digestion. This work will be completed by measurement of elastases I and II activities in infant duodenal contents, especially in milk atopic patients.

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