Macromolecular Absorption in Preterm and Term Infants

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ABSTRACT. Axelsson, I., Jakobsson, I., Lindberg, T., Polberger, S., Benediktsson, B. and Räihä, N. (Department of Paediatrics and Experimental Research, Malmö, and Department of Paediatrics, Umeå, Sweden). Macromolecular absorption in preterm and term infants. Acta Paediatr Scand 78: 532, 1989.

Human α-lactalbumin (α-LA) has been used as a marker for measuring macromolecular absorption. The serum concentration of human α-LA after a human milk feed has been studied in 32 healthy very low birthweight infants (VLBW), fed human milk (gestational age 26–32 weeks) and in 56 term, breast-fed infants, age 3–140 days. At 31 weeks of gestation the serum concentration of human α-LA was more than 10 times higher (mean value 3 000 and median value 2 101 μg/l serum/l human milk/kg body weight, $n=11$) than in the term infants aged 3–30 days (mean value 257 and median value 152, $n=29$). The serum concentration of α-LA decreased with increasing maturity in the VLBW-infants. At a postconceptional age of 37 weeks the values were similar (mean value 200 and median value 99, $n=8$) to those found for term infants during the first month. In the term infants a decreasing absorption of α-LA was found with increasing postnatal age. *Key words: macromolecular absorption, human α-lactalbumin, preterm infants, infancy.*

The transmission of macromolecules across the gut epithelium in different mammals is a well-known phenomenon (1). In experimental animals a period of macromolecular permeability is seen during the neonatal period. This is followed by a considerably reduced transmission (1, 2), which has been called "gut closure" and is considered to represent intestinal maturation, affecting the mucosal barrier (1, 3).

Clinical studies in man have shown that the intestine of newborn infants, especially of preterm infants, may have a higher capacity for absorption of macromolecules compared to the mature adult intestine (4–6).

Several methods have been used to study macromolecular permeability in man. Marker proteins such as bovine serum albumin, ovalbumin and cow's milk proteins have been used (4, 7, 8). When serum concentrations of heterologous proteins are measured, local intestinal and systemic immune responses must be considered. Nonmetabolized compounds, such as PEG (polyethylene glycol) molecules have also been used as markers, but have been shown to be absorbed by the gut by different mechanisms when compared to food proteins (9).

We have developed a competitive radioimmunoassay method for analysis of human α-lactalbumin (α-LA) in serum samples (10), in relation to intake of a human milk test meal. By using a human protein as a marker, local intestinal and systemic immune responses can be avoided.

In the present study we report data on the absorption of human α-LA in VLBW-infants during postnatal development and in term breast-fed infants at different ages. Moreover, the transfer of the protein from mother to infant through the placental barrier has been investigated.

PATIENTS AND METHODS

Blood samples were collected from a catheter in the umbilical vein in 82 VLBW-infants (birthweight <1 500 g, gestational age 24–33 weeks) immediately after birth, before oral feeding with human milk was started. All the infants were appropriate for gestational age. As a routine all infants with a birthweight <1 500 g received an umbilical vein catheter. During the first days of life all infants received intravenous glucose in addition to breast-milk. Of the 82 infants, 32 (gestational age 26–32 weeks) were recruited to a study when they were able to take an oral volume of 170 ml human milk per kg per day, and when intravenous therapy was terminated. They had no physical abnormalities or obvious disease.

From the 32 VLBW-infants a total of 90 venous blood samples (0.5 ml) were collected (2.5 hours after a human milk feed) bi-weekly until term age was reached. Four infants dropped out, because of feeding problems, suspected necrotizing enterocolitis, hypercalcemia or pneumonia which required respiratory assistance.

Umbilical cord samples were obtained from 9 term infants. Blood samples were further obtained at different ages (3–30 days, 31–60 days, 61–140 days), from 56 term breast-fed infants (1–2 hours after a human milk feed). Serum samples were stored at −20°C until analyzed.

Ten ml samples of human milk were collected from the daily milk given to the VLBW-infants. Forty-four out of 93 milk samples were fortified with human milk protein (11). The milk samples were stored at −20°C until analyzed.

Sixty-one 8-hour urine samples were collected into plastic bags on the same days as the serum samples were taken. The urine samples were frozen at −20°C until analyzed.

A competitive radioimmunoassay was used for analysis of α-LA in the serum and urine samples (10). The concentration of α-LA in human milk was determined by electroimmunoassay (12) in 1% agarose with 3% polyethylene glycol (MW.6000). A barbital-sodium-barbital buffer pH 8.6 containing 0.002 M calcium lactate was used. The antibody concentration in the gel was 1–2.5%.

Statistics. The relationship between the degree of maturity and content of α-LA in serum was tested by Spearman's rank order correlation coefficient (r_s).

The study was approved by the Ethics Committee, University of Lund, Sweden.

RESULTS

The mean concentration of α-LA in serum from the umbilical vein, in the 82 VLBW-infants (32 μg/l, range 5–260) was similar to that found in 9 cord blood samples from term infants (mean value 35 μg/l, range 22–72 μg/l).

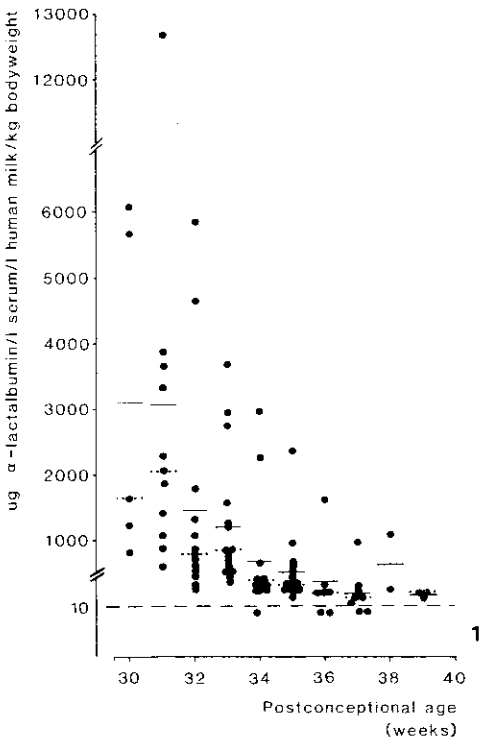
The mean and median concentrations of α-LA in serum (expressed as μg/l serum/l human milk/kg body weight) was highest in the most immature infants and decreased with increasing maturity (Fig. 1). The relationship between serum α-LA level and the postconceptional age was statistically significant ($r_s=-0.69$, $p<0.001$). At 31 weeks of gestation the serum concentration was more than 10 times higher (mean value 3 000, median value 2 101) than in term infants aged 3–30 days (mean value 257, median value 152). When the serum concentration of α-LA was expressed in relation to the amount of α-LA given (μg/l serum/g α-LA/kg body weight) (Fig. 2), there was also a significant relationship between maturity and concentration of α-LA in the serum ($r_s=-0.64$, $p<0.001$).

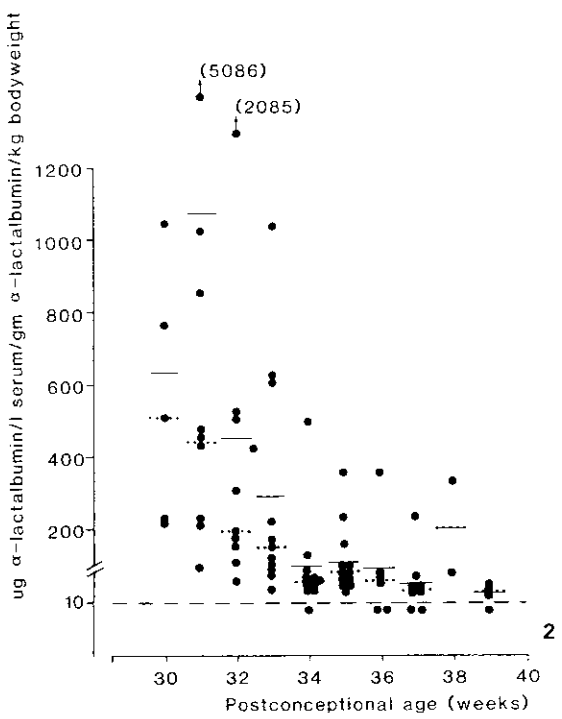
Human α-LA could also be detected in the urine from the VLBW-infants and the highest values were found in samples from the most immature infants (Fig. 3) ($r_s=-0.42$, $p<0.01$).

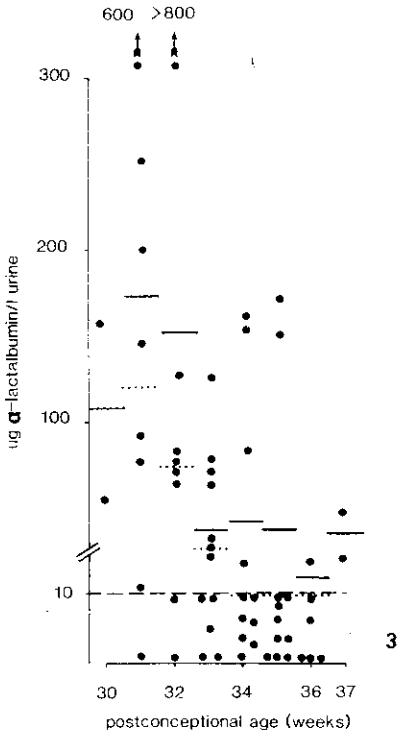
Fig. 4 shows that absorption of α-LA also is found in term infants, and that the serum concentration decreases with increasing postnatal age.

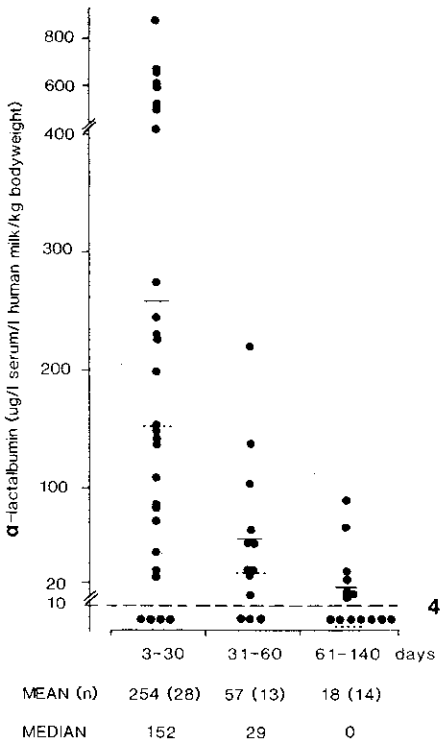
DISCUSSION

With the radioimmunoassay technique immunogenically active α-LA is measured. Thus it might be possible that even small fractions of the molecule are analyzed. To make sure that serum content of the whole molecule α-LA (MW≈14 000) is measured, a gel filtration was done (13) comparing purified α-LA with infant's serum (known to contain α-LA).









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Peak concentration of α-LA occurred in the same fractions, i.e. at the same molecular weight in both purified α-LA and in infant's serum.

The results of this study demonstrate that VLBW-infants given equal volumes of human milk have considerably higher serum concentrations of α-LA at 30–31 weeks postconceptional age than term infants during the first month of life. The serum concentrations of α-LA in the VLBW-infants at 37 weeks are similar to those found in term breast-fed infants aged 3–30 days. Thus, in both VLBW-infants and in term infants the serum concentrations of α-LA decrease with increasing postnatal age.

The most immature VLBW-infants have the highest values of α-LA in urine, indicating a high clearance of the protein from the blood. With advancing maturity the urinary concentrations became successively lower, i.e. the same pattern as was found in serum.

The small concentration of α-LA in the umbilical vein samples are most likely of maternal origin. Serum from pregnant women has been found to contain high levels of α-LA (10). These observations suggest that the protein can pass the placental barrier to some extent.

The amount of α-LA found in blood is the result of several factors, such as the gastrointestinal motility, the proteolytic capacity of the gut, the transfer of the protein from the gut lumen to the blood, the excretion or the clearance rate via the kidneys and possibly also via the gut.

In the preterm infant the gastrointestinal motility has been found to be low (14). The protein digestion in the gut lumen and in the epithelial brush border may be less efficient since peptic and duodenal entero-peptidase activities are low and increase with gestation during the last trimester of pregnancy (15, 16). The activity of trypsin seems to be rather well developed even in preterm infants (17), but low concentrations of cathodal elastase have been found in the duodenal juice in early infancy (18). In vitro studies have shown that the rate of hydrolysis of various bovine whey proteins by human cathodal elastase exceed that of human anodal or cathodal trypsin (19). The low concentration of cathodal elastase in early infancy can be a factor that contributes to the high degree of macromolecular absorption especially in the preterm infants, but may also affect the term infants for some time after birth.

The membranous epithelial M-cells seem to be specially adapted to antigen transport to the underlying gut-associated lymphoid tissue (GALT). The lymphocytes in the GALT are (or may be) precursors of the IgA-producing plasma cells (20). The differentiation of the cells in this system could be immature in preterm infants resulting in decreased synthesis of IgA on the cell surface. Patients with secretory IgA-deficiency have an increased incidence of circulating antibodies to food antigens, suggesting an increased permeability of the intestine (7).

These facts, together with the finding that the concentration of serum α-LA is related to a feeding episode (10), indicate that the high α-LA serum concentration in VLBW-infants is a result of a high degree of macromolecular absorption from the immature gut.

Studies on gut permeability in various mammals have shown an increased absorption of macromolecules during the perinatal period (1). The absorption of bovine β-lactoglobulin

Fig. 1. Concentration of human α-lactalbumin in serum, related to the amount of human milk given per meal, in 32 VLBW-infants in relation to postconceptional age. ——, mean; · · · ·, median.

Fig. 2. Concentration of human α-lactalbumin in serum, related to the amount of α-lactalbumin given per meal, in 30 VLBW-infants in relation to postconceptional age. ——, mean; · · · ·, median.

Fig. 3. Concentration of human α-lactalbumin in 61 urine samples (μg/l), collected during 8 hours, from 32 VLBW-infants in relation to postconceptional age. ——, mean; · · · ·, median.

Fig. 4. Concentration of human α-lactalbumin in serum (μg/l serum/l human milk/kg body weight) in 56 term breast-fed infants of different ages. ——, mean; · · · ·, median.

is significantly higher in preterm infants when compared to that found in term infants (4). These results are in agreement with the high serum concentration of human α-LA in the youngest VLBW-infants in our study. Other studies on preterm infants give evidence of increased gut permeability to bovine serum albumin (5) and to carbohydrates (21).

Our results indicate that significant absorption of α-LA occurs also in term infants. This agrees with the findings by Eastham et al. (6) who observed high concentrations of serum antibodies to food antigens during the first three months of life.

From a physiological point of view, it is tempting to speculate that the increased absorption of macromolecules in the neonates is a physiological phenomenon. A recent study by David et al. (22) done in preterm infants shows no relation between atopic eczema and preterm birth. Studies on animals indicate that it is easier to induce immune tolerance in younger animals given dietary antigen from birth, and in offspring to mothers fed protein antigen before delivery and during the suckling period (23, 24). In contrast, Strobel & Ferguson (25) postulate that after an antigen feed neonatally, immunological and digestive immaturity prevents the induction of oral tolerance.

Nevertheless, it is important to study the underlying mechanisms responsible for the induction of immunity, since disturbances in this system may be related to a variety of pathological conditions such as hypersensitivity, food enteropathies and autoimmune conditions.

In conclusion, this study gives evidence of a considerable absorption of macromolecules in VLBW-infants. The consequences of this cannot be predicted, and further studies related to this observation seem warranted considering the increasing use of artificial formulas for feeding VLBW-infants.

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