

## Neonatal intestinal lactase activity

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**SUMMARY** The sequential changes in intestinal lactase activity of 40 neonates were measured indirectly from the differential uptake and excretion of lactose and the non-metabolisable disaccharide lactulose contained in formula feeds. A daily decline in urinary lactose:lactulose excretion ratios, reflecting a rise in intestinal lactase activity, followed formula feeding. Percentage decline was related directly to gestation: full term infants displayed a fivefold greater decline in lactosuria than infants with a gestation of 28 weeks during the first 10 days of milk feeding. The difference between lactose:lactulose ingestion and excretion ratios suggests that within five days of starting feeds intestinal hydrolysis of lactose exceeds 98% efficiency, even in very preterm infants.

Intestinal lactase activity is detectable in the fetal gut as early as eight weeks' gestation, occurring coincidentally with the previllous ridges.<sup>1</sup> Measurements of lactase activity from tissue obtained from aborted fetuses, stillbirths, and after unfed neonatal deaths have been reported.<sup>2</sup> Mucosal enzyme activities rise gradually between eight and 34 weeks' gestation and then more rapidly to term. Using indirect methods, a postnatal rise in lactase activity after beginning oral feeding in both preterm and full term newborn has been described.<sup>3,4</sup>

Such findings are reflected by the lactosuria observed during the neonatal period. A proportion of unhydrolysed lactose may be passively absorbed and excreted intact in the urine. Although the degree of lactosuria seems to be inversely related to gestational age at birth, and hence may mirror mucosal lactase activity,<sup>5</sup> the capacity of the neonatal gut to absorb disaccharide intact is also affected by its permeability characteristics.<sup>6</sup>

The use of the unmetabolisable reference disaccharide lactulose,<sup>7</sup> composed of fructose and galactose, offers a method of studying sequential changes in intestinal lactase activity as reflected by the quantity of lactose compared with lactulose occurring in the urine of infants fed milk containing these two enzymes. Both sugars have the same molecular weight (342 daltons) and size (0.52 nm) and share the same pathways of passive uptake. Less than 5% of oral loads of lactulose are absorbed in the newborn.<sup>6,8</sup>

The aim of this study was to follow the sequential change in intestinal lactase activity in a group of newborn infants being fed formula during the first

10 days of life, examining in particular its relation to postconceptional and postnatal ages.

### Patients and methods

Forty newborn infants were studied for the first 10 days of life. All were patients of a single maternity hospital in Newcastle and received standard neonatal care throughout the study, which was performed with the informed consent of the parents and approval of the local ethical committee. The weights of the infants ranged from 760 to 4360 g and gestational ages from 27 to 42 weeks at birth.

All infants received an artificial milk formula containing 7 g lactose and 200 mg lactulose per 100 ml feed (lactose:lactulose ingestion ratio of 35:1). This lactulose content is well within that already present in several ready to feed formulas,<sup>9</sup> and no adverse effects were observed. Those babies whose oral feeding was delayed because of prematurity or respiratory disease received 10% dextrose intravenously until oral feeding began. Milk feeds were given by orogastric tube or bottle according to the infant's gestation and condition, but no baby's first feed was delayed longer than four days after birth. No infant suffered severe birth asphyxia. Seven infants required assisted ventilation for respiratory distress syndrome and 23 phototherapy for hyperbilirubinaemia.

A dual marker steady state method of study was used.<sup>6,8</sup> Infants received regular and continuous test feeds. After 24 hours, when a steady state of sugar input and output had been reached, a random urine sample was collected daily for the first two weeks of

life from the preterm infants and until discharge from hospital from term infants. Urine samples were stored at  $-20^{\circ}\text{C}$  with 0.1 ml sodium merthiolate until analysis. Such a method, using lactulose and mannitol as markers, has been validated in catheterised infants, in whom it was shown that a steady state of marker input and output is reached within five times the half life of each marker (20 hours).<sup>8</sup>

Disaccharide concentrations in urine specimens were assayed by gas-liquid chromatography, using turanose as an internal standard.<sup>10</sup> Trimethylsilylation of sugars was performed before chromatography to produce single derivatives of lactulose and turanose and  $\alpha$  and  $\beta$  anomers of lactose. Concentrations were determined by comparison of the peak heights of test sugars with standards after summing the lactose peaks. Results were expressed as a urinary lactose:lactulose excretion ratio.

Multiple regression analysis was used to measure the trend in urinary lactose:lactulose excretion ratios after the start of feeding by fitting parallel trends for each infant and allowing for missing data. This method assumed that the plots for the ratios against days after starting feeds ran parallel. The effect of gestational age on the ratios was determined by linear regression for each day after starting feeds.

The significance of this slope indicated whether or not the two were related and the magnitude of the slope the mean change in ratios for each extra week of gestation.

## Results

Two hundred and twenty one urine specimens were collected from days 1–13 after starting feeds and analysed. The results for days 9–13 were combined to make sufficient numbers for analysis.

There was no significant relation between initial urinary lactose:lactulose excretion ratios and post-conceptual age and weight. They ranged from 4.5 to 0.35 (mean 1.3), representing 7.7- to 100-fold differences from the ingestioun ratio of 35.

The Figure shows the results broken down into three gestational groups—infants of 28, 34, and 40 weeks' gestation. The results are expressed as a percentage decline in urinary lactose:lactulose excretion ratios from initial values plotted against days after starting milk feeds. All three groups showed a decline in ratios during the first five days after the onset of milk feeds. Thereafter, the most mature infants (40 weeks' gestation) showed a continued fall in ratios (eightfold between days 1

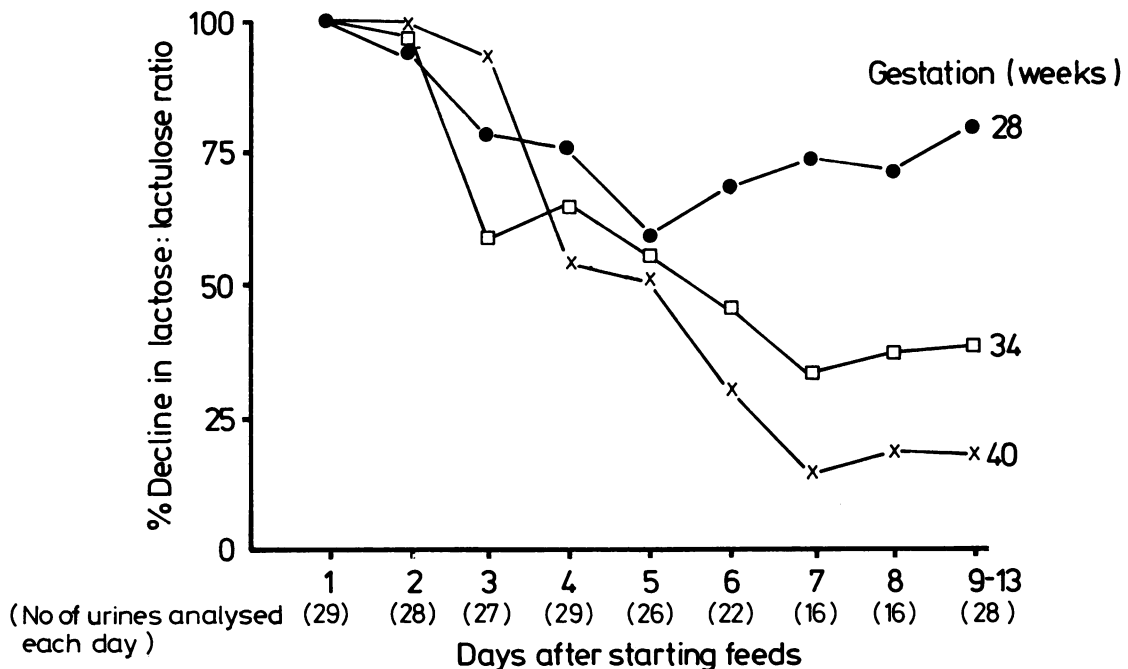


Figure Percentage decline in urinary lactose:lactulose excretion ratios after milk feeding in infants of 28, 34, and 40 weeks' gestation at birth. There was a significant trend in ratios with gestational age from day 7 onwards ( $p \leq 0.01$ ).

and 7), and the most premature (28 weeks' gestation) none, with a persistence of appreciable lactosuria. Infants of 34 weeks' gestation showed a decline in lactose:lactulose ratios intermediate with their less and more mature colleagues. The difference between groups was significant from seven days onwards ( $p < 0.01$ ).

## Discussion

We have shown a rise in intestinal lactase activity after milk feeding in the newborn: the percentage increase is related directly to gestation, which is most pronounced in the term infant and least in the preterm infant.

It has been suggested that initial lactase activity is a function of postconceptional age.<sup>2, 13</sup> Although our method does not allow the measurement of lactase activity before the onset of milk feeding, there was no significant relation between urinary lactose:lactulose excretion ratios and gestation immediately after the onset of milk feeding or during the five following days (Figure). Infants of all gestations showed a sequential rise in lactase activity during this period, and only thereafter was there a significant difference between gestational groups.

These findings contrast with those of Antonowicz and Lebenthal, who found a direct relation between unfed mucosal lactase activities and increasing gestation,<sup>2</sup> but accord with those of Boellner *et al*, who studied the lactose tolerance of preterm and term infants after feeding,<sup>3</sup> and Mayne *et al*, who studied the jejunal fluid sucrose:lactase ratios.<sup>4</sup>

Our findings suggest that the lactase activity of the newborn is equal to the immediate demands of milk feeding but that after five days, when the lactose load has risen appreciably, only the full term infant displays a complete capacity to use this sugar.

Human<sup>13</sup> and animal studies<sup>14</sup> have suggested that intestinal lactase activity is begun or stimulated by milk feeding. Within 24 hours of starting milk feeds all our infants displayed highly significant differences between lactose:lactulose excretion and ingestion ratios, indicating considerable hydrolysis of lactose during this time. On the first day after starting oral feeds the mean excretion:ingestion ratio was 0.04 (1.3:35) and within five days had dropped further to 0.02 (0.7:35) ( $p < 0.0001$  in both instances), suggesting hydrolysis of 98% of ingested lactose. These conclusions contrast with those obtained using breath hydrogen measurements alone to assess lactose utilisation, which suggest significant malabsorption of lactose in preterm<sup>15</sup> and term infants.<sup>16</sup> They conform, however, to the recent finding, using [13C] labelled lactose, that lactose absorption in the small intestine is nearly

complete in the full term infant fed formula.<sup>17</sup> In clinical practice lactose utilisation is not regarded as a major practical constraint to the growth of the newborn, and the indirect measurements of unhydrolysed lactose described both here and in [13C] studies may simply signify the more than adequate amounts of lactose ingested by the newborn.

It is not possible, using this method, to show whether such changes are the direct effect of milk feeding. Analysis of our results by postnatal age simply moves the values for the most preterm infants (as shown in the Figure) to the right. To answer this question it would be necessary to study infants denied milk feeding and those receiving a non-lactose containing formula.

Our findings reflect the degree of maturation of mucosal function of infants of different gestational ages and the capacity of the neonatal intestinal epithelium to respond to the demands of enteral nutrition. They accord with those previously reported describing the maturation in intestinal permeability to lactulose that follows feeding in the newborn.<sup>6</sup> Preterm infants of less than 34 weeks' gestation exhibited a higher intestinal permeability than those more mature at birth, and all showed an appreciable decline in lactulose uptake during the first week of milk feeding.

The effect of such changes in permeability on the uptake of intact lactose is eliminated by the comparison of its uptake with that of the non-metabolisable reference marker lactulose. Both are handled by the body in the same ways, from ingestion to excretion, except for their differing susceptibility to hydrolysis by lactase. Both disaccharides traverse the intestinal wall by simple passive diffusion and share equal volumes of distribution and rates of renal clearance.<sup>11, 12, 18</sup>

It is unlikely that selective breakdown of lactose by micro-organisms in the gastrointestinal tract accounts for our findings. Colonisation of the healthy neonatal small bowel, the site of passive disaccharide absorption, is negligible,<sup>19</sup> and on reaching the large bowel both disaccharides are equally subject to breakdown by bacterial flora.<sup>16, 17, 20</sup>

The dual marker steady state method offers a simple, convenient, non-invasive way of studying sequential changes in intestinal lactase activity in infant fed formula. Our findings suggest mucosal lactose hydrolysis approaches optimal efficiency soon after the onset of milk feeding, even in the very preterm infant, which displays a persisting lactosuria representing no more than the 2% of lactose that escapes hydrolysis in the small intestine.

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