

Ontogeny of pancreatic exocrine function

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Abstract

Exocrine pancreatic proteolytic activity, determined by serial measurement of faecal chymotrypsin concentration, was investigated in 21 preterm infants (23-32 weeks' gestation) during the first 28 days of life. The overall chymotrypsin concentration range was similar to that already described in term infants showing that pancreatic chymotrypsin secretion is equally well developed at birth in the preterm infant. A chymotrypsin concentration peak, seen in term infants at 4 days, did not occur in this study until day 8, suggesting a slower initiation of pancreatic exocrine function in the preterm infant. Median faecal chymotrypsin concentrations, calculated for each baby using data from stools passed between day 2 and day 12 of life, were significantly lower in infants who were small for gestational age when compared with those who were an appropriate size for gestational age. The lower chymotrypsin concentration in infants who were small for gestational age suggests a deleterious effect of intrauterine growth retardation on pancreatic exocrine function which may be a factor in limiting postnatal catch up growth.

The effect of postnatal feeding and other environmental factors on the ontogeny of the gastrointestinal tract is clearly important for survival of the preterm neonate. This is even more important in preterm infants with intrauterine growth retardation who may be less able to absorb nutrients than appropriately grown infants,¹ and who may have suboptimal growth throughout life. Such children are three times more likely than non-growth retarded babies to die during the first year of life.² It has been suggested that by compromising nutrient digestion and absorption, intrauterine growth retardation may play an important part in subsequent poor postnatal growth.³ While few data are available from studies in humans, animal investigations have shown a clear association between intrauterine growth retardation and depression of exocrine pancreatic function.⁴ Severely affected infants may therefore suffer an additional disadvantage through relative pancreatic exocrine insufficiency.

Intraduodenal tests are the most accurate method for determining exocrine pancreatic function,⁵ but they are clearly inappropriate for repeated investigation of preterm neonates being invasive, expensive, and technically complex. In contrast, faecal chymotrypsin measurement is non-invasive, cheap and simple,⁶ and it

is therefore ideal for repeated use in longitudinal studies. A recent study from this department has shown that there is a strong positive correlation between apparent pancreatic chymotrypsin and trypsin secretion rates after intravenous pancreozymin-secretin and faecal chymotrypsin concentrations.⁷ We have therefore used serial measurements of chymotrypsin concentrations in stool to investigate the development of pancreatic secretory capacity over the first few weeks of life in preterm neonates who were either appropriate (AGA) or small for gestational age (SGA).

Subjects and methods

Twenty one preterm infants of 32 weeks' gestation or less (median gestational age 30 weeks, range 23-32), consecutively admitted to a regional neonatal intensive care unit, were included in the study from birth. The median birth weight was 1210 g (range 590-2100). Fourteen infants were normally grown (AGA), while seven showed evidence of intrauterine malnutrition with weight less than the 10th centile for gestational age (SGA). Gestational ages in the infants in the AGA group (26-32 weeks) and SGA group (23-32 weeks) were similar ($p > 0.1$). The assessment of gestational age was based on clinical examination performed on the first day of life,⁸ together with reference to routine early antenatal ultrasonic measurements.⁹

All infants received milk volumes related to their body weight: 60 ml/kg for the first two days of life, increasing by 30 ml/kg every two days to a total of 150 mg/kg. Five received only expressed breast milk; 16 were given breast milk followed by Premium (Cow and Gate) or Pregestimil (Mead Johnson). Two babies were given parenteral nutrition during part of the study period. Two developed necrotising enterocolitis during the four week study period. The results from infants with necrotising enterocolitis are included in this report but excluded from the SGA-AGA comparison.

STOOL COLLECTION AND ANALYTICAL METHODS

Stool specimens were collected daily during the first three weeks of life and then twice during the final week. Occasional days were omitted in most babies but all babies were studied for a minimum of 10 days. Seven patients were transferred back to referring hospitals before completion of four weeks. Specimens were collected in polystyrene Fecon stool containers, and stored at -20°C until analysed. Chymo-

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tryptic activity was measured using the Haverback kinetic potentiometric method.⁶ Details of the procedure as it was used, and performance data for the method, have been published previously.⁷

STATISTICAL EVALUATION

The results were evaluated using non-parametric tests. Spearman's rank correlation coefficient was used to determine the degree and significance of association between two measurements; the significance of the differences between groups was determined with the Mann-Whitney U test.

Results

Faecal chymotrypsin concentrations in the two babies in AGA group who had necrotising enterocolitis were clearly different from the rest of the patients studied, and they are therefore considered separately.

BABIES WITHOUT EVIDENCE OF NECROTISING ENTEROCOLITIS

A total of 305 random stool specimens were obtained from the 19 preterm neonates without necrotising enterocolitis studied. For any individual patient, results showed a wide scatter over the four week period but only 22 (7%) specimens produced a result below the lower reference limit of 120 μg chymotrypsin/g stool previously determined for neonates greater than 36 weeks' gestation.¹⁰ Almost all of the low results occurred in the first few days of life with only one baby producing results below this limit after day 5.

BABIES WITH NECROTISING ENTEROCOLITIS

Fourteen (47%) of 30 results in the two babies who developed necrotising enterocolitis were below the lower reference limit of 120 μg chymotrypsin/g stool. One infant receiving breast milk had a low result on day 2, developed necrotising enterocolitis on day three of life, and was then intravenously fed until day 19. Results above 120 $\mu\text{g}/\text{g}$ stool were only seen from day 12 onwards. The second child was also fed with breast milk until signs of necrotising enterocolitis were noted on day 13, after which parenteral nutrition was given for six days. Daily stool chymotrypsin concentrations were below the lower reference limit up to day 5 and from days 8 to 13; concentrations were above the reference limit on day 6 (160 $\mu\text{g}/\text{g}$), day 7 (360 $\mu\text{g}/\text{g}$), and after day 14.

The random stool data from both the infants in the AGA and SGA groups (excluding the two babies with necrotising enterocolitis) were grouped into 24 hour periods (fig 1) and median values determined. The median concentration in babies in the AGA group increased steadily over the first week, reaching a peak value of 700 $\mu\text{g}/\text{g}$ stool by day 8, followed by a decline over the next two to three days, falling to 320 $\mu\text{g}/\text{g}$ stool by day 12. The median concentration in babies in the SGA group also increased over the

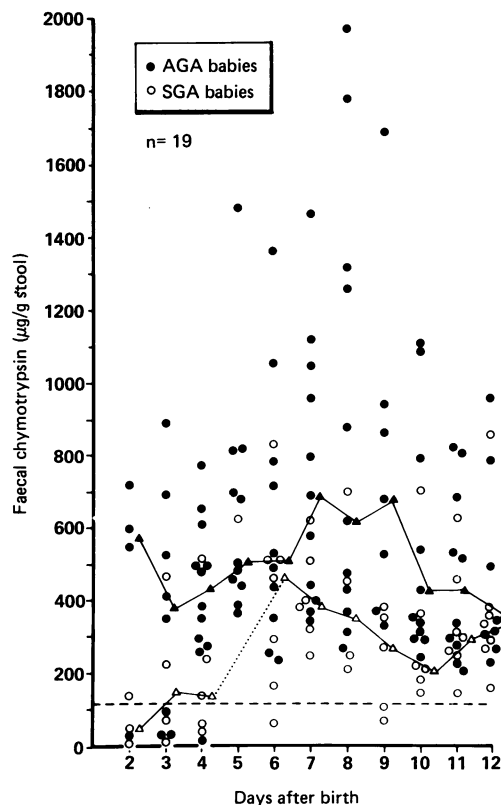


Figure 1 Association between faecal chymotrypsin and time from birth. Random stool chymotrypsin concentrations were grouped into 24 hour periods for days 2–12 from birth. The data from the two babies with necrotising enterocolitis were omitted. The median chymotrypsin concentrations (indicated by triangles) for each 24 hour period in infants in both the AGA and SGA groups are indicated by the continuous lines. The horizontal dashed line shows the lower limit of the observed range determined in 25 term babies.¹⁰

first week reaching a lower peak value of 470 $\mu\text{g}/\text{g}$ stool by day 6 followed by a decline and subsequent rise to 320 $\mu\text{g}/\text{g}$ stool by day 12. Faecal chymotrypsin concentrations on days 7 and 8 in infants in the AGA group were significantly higher than those on days 11 and 12. Faecal chymotrypsin concentrations on days 5 and 6 in infants in the SGA group were not significantly higher than those on days 11 and 12.

Median faecal chymotrypsin in relation to gestational age, birth weight, and intrauterine growth retardation

A median faecal chymotrypsin concentration was calculated for each baby using data from all stool specimens passed between day 2 and day 12 of life. The first day was not included because many of the most preterm infants opened their bowels for the first time on the second to the fourth day. The period day 2–12 was somewhat arbitrary and based on the observation that this interval encompassed the period of surge in faecal chymotrypsin and the fact that specimens were obtained for all babies up to day 12 but not thereafter due to transfer back to referring hospitals.

No correlation was found between median faecal chymotrypsin and gestational age ($r=0.28$, $p>0.05$). However, there was a significant positive correlation ($r=0.456$, $p<0.05$) between

median faecal chymotrypsin and birth weight (fig 2). Four babies in the SGA group had birth weights (880–1340 g) within the range of the 12 babies in the AGA group (800–2110 g), but they had significantly lower median chymotrypsin

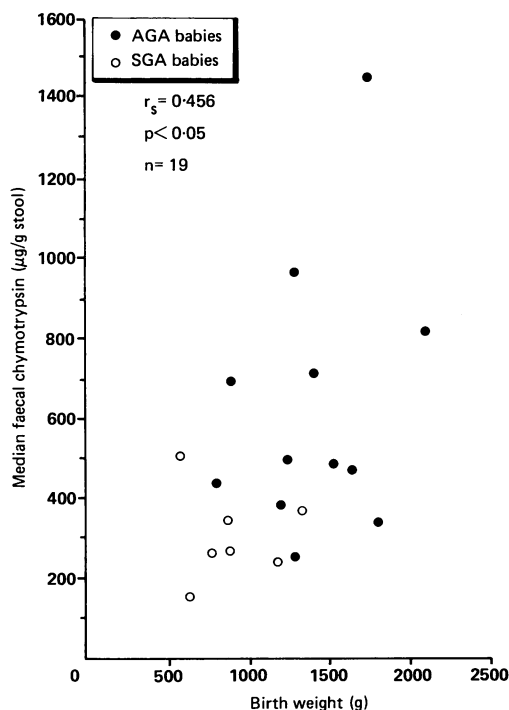


Figure 2 Correlation between faecal chymotrypsin and birth weight. Median faecal chymotrypsin concentrations were calculated for all babies for the period days 2–12 after birth. The median values showed a significant positive correlation with birth weight.

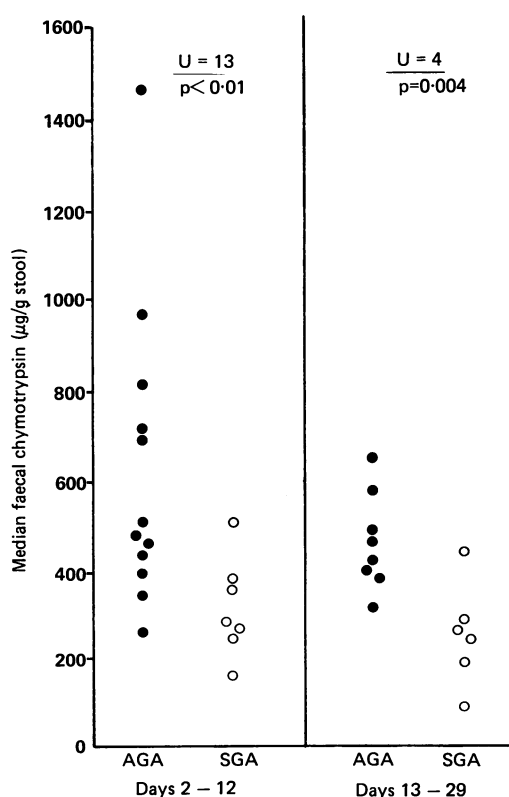


Figure 3 Comparison of faecal chymotrypsin concentrations in babies in the AGA and SGA groups for two periods from birth. Concentrations were significantly lower in the babies in the SGA group compared with those in the AGA group in both periods.

concentrations ($p=0.01$). When median chymotrypsin concentrations between 2 and 12 days in both groups of infants were compared, those in the SGA group were shown to have significantly lower values ($p<0.01$). Median concentrations calculated for the period 13–29 days (fig 3) showed that the differences between the two, now smaller, groups was sustained ($p=0.004$). Two babies who were partly parenterally fed had stool chymotrypsin values within the range observed for the rest of the group.

Discussion

The daily ranges of faecal chymotrypsin concentration determined in this study were similar to those described in term infants, only a few results falling below the term infant reference range¹⁰ during the first four days of life in the babies in the AGA group, and over the first nine days of life in those in the SGA group.

The daily median chymotrypsin concentration for both groups of babies increased steadily after birth reaching a peak at six days and eight days respectively. A similar peak has been reported in term infants but occurred at four days of age.¹⁰ As previously described in term babies a significant positive correlation ($r=0.456$, $p<0.05$) was found between birth weight and faecal chymotrypsin,¹⁰ with babies in the SGA group having significantly lower median chymotrypsin concentrations than those in the AGA group.

There is considerable uncertainty about the ontogeny of pancreatic exocrine function in the human neonate, with the principal enzymes each displaying a unique maturational pattern.¹¹ A previous intraduodenal study of pancreatic function indicated that the preterm neonate of 32 weeks' gestation or more has a significantly lower pancreatic enzyme secretion rate than the term infant.¹² Other workers, however, have claimed that enzyme secretion in preterm babies of greater than 32 weeks' gestation is the same as that at term,¹¹ and with respect to chymotrypsin at least, our results would support this.

Our study suggests that even premature babies from 23–32 weeks' gestation, a group not previously studied, secrete chymotrypsin in quantities similar to term babies, but may take a few days longer to fully establish secretory activity. Antonowicz and Lebenthal failed to detect enterokinase in postmortem fetal small bowel mucosa before 26 weeks' gestation,¹³ but our youngest subject with a gestational age of only 23 weeks showed faecal chymotrypsin concentrations within the reference range for term infants,¹⁰ indicating indirectly the presence of adequate enterokinase activity.

The surge seen in faecal chymotrypsin over the first few days of life has also been noted in intraduodenal studies of pancreatic exocrine function,¹² gastric acid secretion,¹⁴ and plasma gastrin and enteroglucagon.¹⁵ It is possible that the surge in chymotrypsin is a consequence of the secretion of regulatory peptides in response to food in the gut.¹⁶ Their effects may trigger a cascade of developmental changes including modulation of pancreatic exocrine function. The importance of enteral feeding in this

mechanism is indicated by the observation that surges in gastrointestinal hormones seen in preterm infants given bolus milk feeds were not seen in another group of infants who did not receive enteral feeds.¹⁶ Changes in hormone concentrations do not occur in infants who have never been fed.¹⁷ Deficient plasma clearance or the relatively large endocrine cell mass in the developing gut may explain why plasma concentrations of regulatory peptides reach much higher levels in preterm infants than adults. Such high concentrations may be necessary to induce receptor activity and the functional effects in target tissues.¹⁶

Two babies with subnormal faecal chymotrypsin measurements subsequently developed necrotising enterocolitis. The significance of this finding is uncertain, but suggests that impaired pancreatic proteolysis may be a contributory factor in the pathogenesis of necrotising enterocolitis. It is tempting here to draw an analogy with 'pig-bel', a fatal necrotising enterocolitis that occurs in the New Guinea highlands, when *Clostridium perfringens* infection coincides with the ingestion of large quantities of trypsin inhibitors in sweet potato.¹⁸ Alternatively, necrotising enterocolitis predates clinical symptoms, inhibiting pancreatic secretion while the baby is still apparently well.

The exocrine pancreas has a high rate of protein synthesis and it is not surprising that protein deprivation adversely affects function. Malnourished children have been shown to have severely reduced enzyme output with normal volume of secretions and bicarbonate output.¹⁹ Some children have shown little or no recovery of pancreatic exocrine function after nutritional repletion,²⁰ and malnutrition in childhood may result in irreversible pancreatic atrophy. Animal experiments have demonstrated that intrauterine growth retardation can also lead to impairment of pancreatic function. Studies on pregnant rats, for example, have shown that a reduction in uterine blood flow leads to decreased pancreatic weight and amylase and lipase content, but with unaffected concentrations of proteases in the rat pups.⁴ Similar findings have been reported in rat pups both from dams fed an inadequate diet during pregnancy²¹ and when malnourished in the early neonatal period.²² By showing that infants who are small for gestational age have significantly lower faecal chymotrypsin concentrations than infants of similar weight who are of appropriate size for gestational age, our study provides evidence for the first time that inadequate nutrition in utero may also have an adverse effect on pancreatic function in the human infant, which persists for at least the first four weeks of postnatal life. Although the

consequences of such an effect remain unknown, for the infant born with intrauterine growth retardation and faced with the necessity of catch up growth, it could represent a significant handicap and highlights the cardinal importance of appropriate nutrition.

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