Intestinal permeability in relation to birth weight and gestational and postnatal age

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Objective: To determine the relation between intestinal permeability and birth weight, gestational age, postnatal age, and perinatal risk factors in neonates.

Study design: Intestinal permeability was measured by the sugar absorption test within two days of birth and three to six days later in preterm and healthy term infants. In the sugar absorption test, the urinary lactulose/mannitol ratio is measured after oral ingestion of a solution (375 mosm) of lactulose and mannitol.

Results: A first sugar absorption test was performed in 116 preterm (26–36 weeks gestation) and 16 term infants. A second test was performed in 102 preterm and nine term infants. In the preterm infants, the lactulose/mannitol ratio was not related to gestational age ($r = -0.09, p = 0.32$) or birth weight ($r = 0.07, p = 0.43$). The median lactulose/mannitol ratio was higher if measured less than two days after birth than when measured three to six days later (0.427 and 0.182 respectively, $p < 0.001$). The lactulose/mannitol ratio was higher in preterm infants than term infants if measured within the first 2 days of life (0.404 and 0.170 respectively, $p < 0.001$), but not different three to six days later (0.182 and 0.123 respectively, $p = 0.08$). In multiple regression analysis of perinatal risk factors, only umbilical arterial pH correlated with the lactulose/mannitol ratio in preterm infants less than 2 days of age ($T = -1.98, p = 0.05$).

Conclusions: In preterm infants (26–36 weeks gestation), intestinal permeability is not related to gestational age or birth weight but is higher during the first 2 days of life than three to six days later. It is higher in preterm infants than in healthy term infants only if measured within two days of birth. This suggests rapid postnatal adaptation of the small intestine in preterm infants.
tions of lactulose and mannitol were measured (in mmol/mol ficient. To evaluate the influence of perinatal factors on 
parametric Wilcoxon signed ranks test for paired data. Corre-
the difference between the median L/M ratio 
Data were analysed with SPSS 9.0 (SPSS Inc, Chicago, Illinois, USA). They are given as median values and range unless indi-
committee of the Isala Clinics, Location Sophia, Zwolle.

Methods
The SAT was performed as previously described,11 with slight modifications related to the study population. The test 
solution was prepared under sterile conditions. Instead of a 
hyperosmolar solution, a less hyperosmolar (375 mosm) solu-
tion was used by omitting sucrose from the test solution. The test solution contained 250 mg lactulose and 100 mg manni-
tol per 5 ml water. Fasting before the test was not mandatory. 
The test solution was instilled by nasogastric tube (2 ml/kg body weight). Urine was then collected for six hours. Chlorhexitidine digluconate (0.1 ml of a 20% solution) was 
added to the urine as a preservative. The urine volume was 
measured and samples were stored at −20°C. The concentra-
tions of lactulose and mannitol were measured (in mmol/mol creatinine) in the samples by gas chromatography as previously described,12 and the L/M ratio was calculated. Reference values for the L/M ratio with the hyperosmolar test solution in both older infants and adults are 0–0.089.1 In infants, complete collection of urine may be difficult. In our 
study, complete urine collection was achieved in 36% of the 
infants. Incomplete urine collection did not seem to influence 
the results of the study, therefore we combined the results for 
infants with and without complete urine collection as long as 
at least 5 ml, the minimum volume required for analysis, was collected.

Study protocol
After informed parental consent, infants were tested within 
two days of birth. A second test was performed three to six 
days after the first test. Perinatal factors, 5 min Apgar score, 
umbilical arterial pH (if available), birth weight, gestational 
age, enteral feeding in ml/kg/day, and the neonatal therapeu-
tic intervention scoring system (NTISS)17 were recorded. The study protocol was approved by the hospital medical ethics 
committee of the Isala Clinics, Location Sophia, Zwolle.

Statistical analysis
Data were analysed with SPSS 9.0 (SPSS Inc, Chicago, Illinois, USA). They are given as median values and range unless indicated otherwise. The difference between the median L/M ratio 
on the first test and the second test was tested with the non-
parametric Wilcoxon signed ranks test for paired data. Corre-
lations were determined using Pearson’s correlation coef-
icient. To evaluate the influence of perinatal factors on 
intestinal permeability, multiple regression analysis was performed. A two sided p value < 0.05 was considered signifi-
cant.

RESULTS
Preterm infants
In 116 preterm infants, 68 boys and 48 girls, the SAT was per-
formed within two days of birth, at a median postnatal age of 
28.5 hours (range 4–48). The median gestational age was 31 
weeks and 2 days (range 26 weeks and 3 days to 36 weeks and 
4 days), and birth weight was 1425 g (range 665–2720). In 102/116 (88%) preterm infants, a second SAT was performed at a postnatal age of 141 hours (range 91–180). In 14 infants, 
the second SAT could not be performed for reasons such as 
early transfer to another hospital, failure to collect urine, or 
death of the infant. Urine was collected for all six hours in 
41/116 (35%) preterm infants in the first test and 37/102 (36%) preterm infants in the second test. 

The L/M ratio of the first SAT was not related to gestational age 
r = −0.09, p = 0.32; fig 1), birth weight (r = 0.07, p = 0.43), or postnatal age (hours) at which the first test was performed (r = −0.13, p = 0.15). If the preterm infants are 
divided into two groups (< 34 weeks and > 34 weeks gestation), no differences between the two groups were found 
in the L/M ratio measured within two days of birth or three to 
six days later.

The L/M ratio of the first SAT was higher than that of the 
second SAT (0.427 and 0.182 respectively, p < 0.001; fig 2). The decrease in L/M ratio was due to both increased 
permeability to mannitol (p < 0.002) and, to a lesser extent, 
decreased permeability to lactulose (p < 0.02). The L/M ratio 
of the second SAT was not related to the postnatal age at which the second SAT was performed (r = −0.025, p = 0.80).

If “critically ill” preterm infants, defined as infants on a 
ventilator, suffering from asphyxia, and/or born small for 
gestational age, were compared with “healthy” preterm infants, no differences in intestinal permeability were found 
either at the first test or the second test. The NTISS did not 
correlate with the L/M ratio at either the first SAT (r = −0.13, p = 0.17) or the second (r = −0.09, p = 0.38). The NTISS at 
the first SAT (15.6, range 1–27) was higher than the NTISS at the second SAT (12.9, range 0–25) (p < 0.001). However, the decrease in the L/M ratio was not related to the decrease in the 
NTISS (r = −0.07, p = 0.50).

In multiple regression analysis of perinatal factors, only 
umbilical arterial pH correlated somewhat with the L/M ratio 
measured within two days of birth (T = −1.98, p = 0.05).

Preterm versus term infants
Sixteen term infants were tested within two days of birth. In 
ine of them, a second SAT was performed three to six days
Beach lactulose and mannitol, in neither that study nor the one of the osmolarity of the feeds was unchanged by the addition of that may also be due to changes in perinatal treatment since that gestational age of 28 weeks; intestinal permeability was not different at a postnatal age of 10 days, which is in line with our results from the second test at the postnatal age of 4–7 days. In our study, intestinal permeability was not different in preterm infants exposed to enteral steroids (n = 70) and preterm infants not exposed to enteral steroids (n = 46), both measured within two days of birth and three to six days later. Besides differences in postnatal age, severity of illness may influence intestinal permeability. In critically ill adults, intestinal permeability is increased compared with healthy controls. In contrast with previous studies, we included both “healthy” and “critically ill” preterm infants. However, intestinal permeability was not different in “critically ill” preterm infants (infants on a ventilator, suffering from asphyxia, and/or born small for gestational age) compared with “healthy” preterm infants. Excluding “critically ill” preterm infants from our study did not change the differences found between preterm and term infants. Furthermore, although the NTISS decreased between the first and second test, the decrease was not related to the decrease in intestinal permeability between the first and second test. As mentioned above, differences in perinatal treatment may have affected the results of our study compared with the results of the studies performed in the 1980s.

Several other perinatal factors—for example, the amount and type of enteral feeding—may influence intestinal permeability shortly after birth. At the first test, only 30/116 infants received enteral feeding, whereas 95/102 infants had started enteral feeding at the second test. Although most infants had received enteral feeding for only one or two days at the second test, enteral feeding may account for the rapid decrease in intestinal permeability in preterm infants. In the study of Rouwet et al, intestinal permeability increased between postnatal day 1 and 7, and decreased between day 7 and 14. As enteral feeding was started in all infants after day 7, this may explain the temporary increase in intestinal permeability as stated by the authors. Because many infants in our study were fed a mixture of mother’s milk and preterm formula in the first days of life, we were unable to determine the type of enteral feeding on intestinal permeability. Of the perinatal factors evaluated in our study in preterm infants, only arterial umbilical pH correlated with intestinal permeability measured within two days of birth. A low arterial umbilical pH is thought to reflect an impaired

**DISCUSSION**

In our study, the largest study in neonates, intestinal permeability, measured by the sugar absorption test, in preterm infants less than 2 days old was not related to gestational age or birth weight. However, it was higher in preterm infants than in healthy term infants (> 37 weeks of gestational age) but only if measured within two days of birth. At a postnatal age of 4–7 days, intestinal permeability was not different in preterm infants and term infants. Our findings are in line with the results of Shulman et al. and Rouwet et al. who found that intestinal permeability was not different in infants with a gestational age of < 28 weeks compared with infants with a gestational age of > 28 weeks. However, in the study of Shulman et al., intestinal permeability was not measured before the postnatal age of 10 days. In two other studies, however, intestinal permeability in preterm infants was found to be related to gestational age. Several methodological differences exist between our study and the latter studies. In those studies, the so-called steady state method for the measurement of intestinal permeability was used. The osmolarity of the test solution in intestinal permeability tests is known to influence the test results. Although Weaver et al. state that the osmolarity of the feeds was unchanged by the addition of lactulose and mannitol, in neither that study nor the one of Beach et al. did the osmolarity of the test solution reported. We used the single load method as we have used previously in older children and adults. This method has the advantage that only one (small) dose of the test solution needs to be given. Therefore, the test is also feasible in very sick infants who do not tolerate normal amounts of enteral feeding. Intestinal permeability tests, measuring the ratio of two macromolecules in urine, are less influenced by variables such as gastric emptying and renal excretion. Furthermore, the ratio of two macromolecules in urine depends less on complete urine collection for six hours, which is in accordance with the results of Akram et al. who found no difference in intestinal permeability between a two hour urine collection and a five to six hour urine collection in adults. The results of our study are in line with these findings, as we did not find any difference between preterm infants in whom urine collection was complete and preterm infants in whom urine collection was incomplete but at least 5 ml.

**Figure 3** Lactulose/mannitol (L/M) ratio in 116 preterm infants and 16 healthy term infants measured within two days of birth (test 1). It was significantly higher in the preterm than the term infants (0.404 and 0.170 respectively, p < 0.001).
condition of the fetus shortly before birth. This may influence the condition of the small bowel leading to increased intestinal permeability, which in turn may increase the risk of necrotising enterocolitis. However, to our knowledge, no other studies have looked at arterial umbilical pH in preterm infants in relation to intestinal permeability or necrotising enterocolitis.

Although in preterm infants, intestinal permeability was not related to gestational age, it was higher in preterm infants than in healthy term infants. We hypothesise that the continuous maturation of the intestinal mucosa may decrease intestinal permeability in the last few weeks of pregnancy. In addition, the causes of preterm birth—for example, maternal infection—may play a role in the increased intestinal permeability in preterm infants shortly after birth compared with healthy term infants who are born after elective caesarean section.

In summary, we have shown that intestinal permeability, measured within two days of birth by a single load sugar absorption test, is not related to gestational age or birth weight in preterm infants 26–37 weeks of gestational age. However, it is higher in preterm infants than in term infants during the first 2 days of life, but not different at 4–7 days of age. Intestinal permeability in preterm infants decreases in the first week of life, suggesting that postnatal factors play an important role in the rapid adaptation of the small intestine to the extrauterine circumstances.

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