

Intestinal Permeability and Carrier-Mediated Monosaccharide Absorption in Preterm Neonates during the Early Postnatal Period

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ABSTRACT

Immaturity of intestinal epithelial barrier function and absorptive capacity may play a role in the pathophysiology of intestinal complications in preterm neonates during the early postnatal period. We determined the intestinal permeability and carrier-mediated absorption of monosaccharides in preterm neonates during the first 2 wk after birth. Fifty-nine preterm neonates born between 25 and 32 wk gestation were included within 24 h of birth. Neonates received exclusively parenteral nutrition during the first 7 d after birth; enteral feeding was initiated at d 8. An intestinal permeability-absorption test was performed at 1, 4, 7, and 14 d after birth. The lactulose-to-rhamnose ratio was determined as a marker of intestinal permeability. Urinary excretion percentages of D-xylose and 3-O-methyl-D-glucose were determined as markers of passive and active carrier-mediated monosaccharide absorption, respectively. Intestinal permeability transiently increased between d 1 and 7 in all neonates ($p <$

0.05). Carrier-mediated monosaccharide absorption increased between d 1 and 14 in neonates of 28–30 wk ($p < 0.05$) to the level observed in the neonates of 30–32 wk gestation. In neonates < 28 wk, intestinal permeability at d 7 was higher ($p < 0.05$) and carrier-mediated monosaccharide absorption at d 14 was lower ($p < 0.01$) as compared with neonates ≥ 28 wk. The barrier function of the intestinal epithelium transiently decreases during the first week after birth in preterm neonates who are not enterally fed. Diminished barrier function and low monosaccharide absorptive capacity, particularly in neonates < 28 wk, may predispose these patients to the development of intestinal complications during the early postnatal period. (*Pediatr Res* 51: 64–70, 2002)

Abbreviation

L/R ratio, lactulose/L-rhamnose ratio

Gastrointestinal complications, such as feeding intolerance, necrotizing enterocolitis, and gut-associated sepsis, pose a considerable problem in the care of preterm neonates. At present, necrotizing enterocolitis is the most common surgical emergency and a major cause of death in this patient population (1–3). These intestinal complications occur mostly in the first weeks after birth, suggesting that they result from failure of the immature intestine to adapt adequately to the transition from intrauterine to extrauterine life.

Birth induces two major changes in the luminal environment of the intestine. First, after delivery the neonatal gut is exposed to microorganisms and their products. To prevent host invasion by potentially pathogenic microorganisms, a full barrier function of the intestinal wall is required. Second, the i.v. nutrient supply via the placental-umbilical circulation is interrupted after birth. Con-

sequently, the neonate depends on the gastrointestinal tract for the acquisition of nutrients through the processes of propulsion, digestion, and absorption of ingested food.

The anatomic differentiation of the human fetal gut is already completed by 20 wk gestation (4). However, mature levels of digestive enzyme secretion are not reached until the end of fetal gestation (5, 6). Lactase activity at 34 wk gestation is only 30% of the level in the full-term newborn (7). Furthermore, intestinal motor activity in response to luminal nutrients is not present until 31 wk, and is still immature at 40 wk gestation (8). Thus, the development of various functions of the intestine lag behind its structural development.

Little is known about the development of the barrier function as well as the absorptive function of the human gut. Intestinal barrier function is in part dependent on the close interaction of intact adjacent epithelial cells, which impedes the diffusion of luminal substances. This property of the epithelium can be assessed indirectly by determining the intestinal permeability to orally administered water-soluble molecules, such as lactulose and L-rhamnose (9, 10). It has previously been shown in preterm neonates that the

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permeability changes during the first month after birth, and that it may be affected by enteral nutrition (11–13). However, these studies did not investigate the permeability during the immediate postnatal period, or did not discern between preterm neonates of different gestational ages. The aim of the current study was therefore to investigate the intestinal permeability in preterm neonates born at 25 to 32 wk gestation during the first 14 d after birth. Next, we studied the capacity of the intestinal epithelium to absorb monosaccharides by both passive and active carrier-mediated transport mechanisms, by measuring the urinary excretion of orally administered D-xylose and 3-O-methyl-D-glucose in these neonates. To investigate the influence of enteral nutrients on intestinal permeability and monosaccharide absorption, these variables were studied during a 1-wk period of exclusive parenteral nutrition as well as after the initiation of enteral feeding.

METHODS

Patients and study design. The study was conducted in the neonatal intensive care unit of the University Hospital Maastricht from July 1997 to September 1999, and was approved by the local medical ethics committee. Parents of eligible neonates were informed in detail about the study, and written informed consent was obtained before enrollment.

Preterm neonates admitted to the intensive care unit were eligible for the study if they were born at a gestational age of 25–32 wk and if postnatal age was <24 h. Criteria for exclusion included major congenital anomalies, severe asphyxia (defined as 5-min Apgar score ≤ 3), persistent hypoxemia or respiratory acidosis, and severe hypotension. For each neonate we recorded clinical characteristics at study entry, including gestational age, birth weight, sex, Apgar score at 1 and 5 min, CRIB score (Clinical Risk Index for Babies) (14), presence of asphyxia, mode of delivery, prolonged rupture of the membranes (>24 h), chorioamnionitis, (pre)eclampsia/HELLP syndrome, antenatal administration of corticosteroids, tocolysis, and multiple pregnancy. We furthermore recorded the occurrence of sepsis, defined as clinical signs of sepsis and positive blood culture.

To investigate the influence of enteral nutrients on intestinal permeability and monosaccharide absorption, the study was implemented in two phases. Between d 1 and 7 after birth, neonates received only i.v. fluids, which consisted of 10% glucose/calcium solution for the first day, and of total parenteral nutrition thereafter. In general, initial fluid intake was 80 mL/kg/d and was increased by 20 mL/kg/d up to approximately 150 mL/kg/d. This volume was adapted on the basis of clinical evaluation, urine production, weight measurement, and serum electrolyte values. From d 8 after birth onward, neonates received enteral feeding in addition to parenteral nutrition. Enteral nutrition consisted of either mothers' own milk (obtained and used within 48 h) or full-strength (24 kcal/30 mL) preterm formula (Nenatal, Nutricia, Zoetermeer, The Netherlands), according to the choice of the parents. Enteral feeding was administered as bolus feedings via a nasogastric tube at an initial volume of 12 mL/d, and increased until complete enteral nutrition (approximately 150 mL/kg/d) was achieved. The rate of enteral feeding increment was determined by the attending physician and adapted as required by the infants' clinical

condition. In case of feeding intolerance, as defined by emesis, large gastric residuals, abdominal distension, or ileus, enteral feedings were reduced or withheld until the problem resolved. Nonbloody gastric residuals of <3 mL per 2 h were refed.

Assessment of intestinal permeability and carrier-mediated monosaccharide absorption. To allow the simultaneous evaluation of intestinal permeability and monosaccharide absorption, we conducted a sugar permeability-absorption test at 1, 4, 7, and 14 d after birth. The sugar solution consisted of 8.6 g lactulose (Centrafarm, Etten-Leur, The Netherlands), 140 mg L-rhamnose (Acros Organics, Pittsburgh, PA, U.S.A.), 70 mg D-xylose (Genfarma, Maarssen, The Netherlands), and 140 mg 3-O-methyl-D-glucose (Sigma Chemical Co., St. Louis, MO, U.S.A.) dissolved in 100 mL of demineralized water (425 mosmol/L). Each test day, 2 mL of the sugar solution was administered to the neonate via the nasogastric tube. All urine passed in the next 4 h was collected in an adhesive urine bag (Urinocol Premature, Braun Biotrol, Paris, France). At d 14, no enteral feeding was given in the 2 h preceding and following ingestion of the test solution. These time points were chosen to minimize interference with routine nursery protocols of the neonatal intensive care unit. The complete 4-h urine volume was measured, and a 2-mL aliquot was stored at -80°C until analysis. The sugar absorption test critically relies on a complete urine collection. Therefore, in case urine collection failed in an infant, the sugar absorption test was repeated the next day. If urine sampling failed again, then measurements for the infant at this particular test day were regarded as missing values. Urinary concentrations of lactulose, L-rhamnose, D-xylose, and 3-O-methyl-D-glucose were determined by gas-liquid chromatography as previously described (15). This test is noninvasive, requires only minimal handling, and can be used safely in preterm neonates (16).

The four saccharides used in this test cross the intestinal epithelium via different pathways and are cleared by the kidneys. The percentage of the orally administered dose of each saccharide that is excreted in the urine thus reflects the functional state of the particular intestinal permeation pathway. Lactulose is a disaccharide that crosses the intestinal epithelium by passive diffusion through the paracellular tight junctions. L-Rhamnose is a monosaccharide that crosses the intestinal epithelium mainly by transcellular passive diffusion through aqueous pores. The urinary excretion percentages of lactulose and rhamnose are markers for paracellular and transcellular diffusion, respectively. To correct for nonmucosal factors that may affect the intestinal uptake of these saccharides, including the rate of gastric emptying, intestinal transit time, and renal clearance, the urinary excretion percentages of lactulose and rhamnose were expressed as the L/R ratio. Because nonmucosal factors will affect urinary excretion of both saccharides to a similar extent, the L/R ratio provides a reliable index of the permeability of the intestinal epithelium (9, 10). D-Xylose and 3-O-methyl-D-glucose are monosaccharides that are absorbed by the intestinal epithelial cells via passive and active carrier-mediated transport mechanisms, respectively. The urinary recovery of orally administered xylose and methylglucose are markers for passive and active carrier-mediated monosaccharide absorption, respectively (17, 18).

Measurement of urinary D-lactate concentration. To investigate whether an increase in intestinal permeability to sugar probes was associated with an increase in the permeation of other substances present in the intestinal lumen, we measured the urinary excretion of D-lactate. This stereoisomer of L-lactate is derived from fermentation of unabsorbed carbohydrates by bacteria within the gut lumen, is neither produced nor metabolized by mammalian cells, and is excreted by the kidneys. Therefore, urinary D-lactate excretion is considered to reflect bacterial production and intestinal uptake (19).

Urinary D-lactate was determined at 1, 4, 7, and 14 d after birth. Urine samples were stored in 1-mL aliquots at -80°C until measurement. D-Lactate was measured by means of an enzymatic assay according to a method previously described (20). Briefly, thawed urine was deproteinized with perchloric acid and centrifuged. The supernatant was added to an NAD^+ -glycine-hydrazine solution to form pyruvate hydrazone coupled with the reduction of NAD^+ to NADH at a $\text{pH} < 9.0$. This reaction was catalyzed by the enzyme D-lactate dehydrogenase. NADH was measured spectrophotometrically at 340 nm. The D-lactate concentration (in mM) was expressed relative to the creatinine concentration (in mM) in the urine sample to yield the D-lactate/creatinine ratio.

Data analysis. Data are presented either as median and interquartile range (clinical values) or as mean \pm SEM (biochemical variables). The overall effect in time was determined by a repeated measurements analysis using the GLM procedure in SPSS version 10 (SPSS Inc, Chicago, IL, U.S.A.). However, missing data at several points in time precluded the use of this procedure for the assessment of differences among gestational age groups. Therefore, subsequent comparisons within groups were made using the Wilcoxon signed ranks test. Comparisons among groups were made using the Mann-Whitney *U* test. Statistical significance was defined as $p < 0.05$ (two-sided).

RESULTS

Fifty-nine preterm neonates born between 25 and 32 wk gestation were enrolled. Neonates were stratified for gestational age: 25–27⁺⁶ (group A, $n = 18$), 28–29⁺⁶ (group B, $n = 24$), and 30–32 wk gestation (group C, $n = 17$). Clinical characteristics of the infants are summarized in Table 1. The sugar permeability-absorption test was not successful in all neonates at all four times, owing to one of the following reasons: failure of complete urine collection, failure of chemical analysis, unstable clinical condition, or transfer to another hospital.

Repeated measurements analysis for the group of neonates as a whole demonstrated an increase in the excretion of lactulose with time ($p = 0.014$). Subgroup analysis showed that lactulose excretion did not change significantly during the first 14 d after birth in group A, whereas it significantly increased as compared with d 1 at d 4 ($p = 0.033$) and 7 ($p = 0.006$) in group B, and at d 7 ($p = 0.028$) in group C (Fig. 1A).

Repeated measurements analysis of L-rhamnose excretion demonstrated a decrease with time for the whole group ($p = 0.011$). The reduction in urinary L-rhamnose excretion was statistically significant in group A at d 7 ($p = 0.038$) and 14 ($p = 0.050$), and in group C at d 4 ($p = 0.046$). Rhamnose excretion at d 7 and 14 was significantly lower in group A as compared with the other two groups ($p = 0.009$ and $p = 0.008$, respectively; Fig. 1B).

As a result of these changes in lactulose and rhamnose excretion, the L/R ratio increased with time for the group as a whole ($p = 0.001$). Subgroup analysis demonstrated that the increase in L/R ratio between d 1 and 7 was significant in all three groups ($p = 0.018$, $p = 0.006$, $p = 0.046$ in group A, B, C, respectively). This peak in L/R ratio at d 7 was significantly higher in group A as compared with the other two groups ($p = 0.025$). The L/R ratio declined between d 7 and 14 (Fig. 1C).

Table 1. Clinical characteristics of patients*

Variable	Group A (25–27 ⁺⁶ wk) $n = 18$	Group B (28–29 ⁺⁶ wk) $n = 24$	Group C (30–32 wk) $n = 17$
Birth weight (g)	880 (808–1006)	1050 (795–1240)	1340 (1105–1488)
Female:male (% female)	12:6 (66.7)	11:13 (45.8)	8:9 (47.1)
Apgar score			
1 min	6 (3.5–8)	7 (5–9)	5 (4–8)
5 min	9 (7–9)	9 (8–9)	9 (7.5–10)
CRIB score	1.5 (1–7)	1.5 (1–5)	1 (0.5–1.5)
Asphyxia (%)	16.7	16.7	17.6
Cesarean delivery (%)	38.9	62.5	58.8
Rupture of membranes >24 h (%)	22.2	29.2	35.3
Chorioamnionitis (%)	16.7	4.2	0
(Pre)eclampsia/HELLP (%)	5.6	16.7	5.9
Antenatal corticosteroids (%)	83.3	54.2	47.1
Tocolysis (%)	72.2	37.5	52.9
Multiple pregnancy (%)	27.8	25	35.3
Sepsis (%)			
d 1	5.6	0	0
d 4	5.6	8.3	0
d 7	16.7	25	6.7
d 14	27.8	28.6	27.3

* Values are percentage of patients except for birth weight, Apgar score, and CRIB score, which are median (interquartile range).

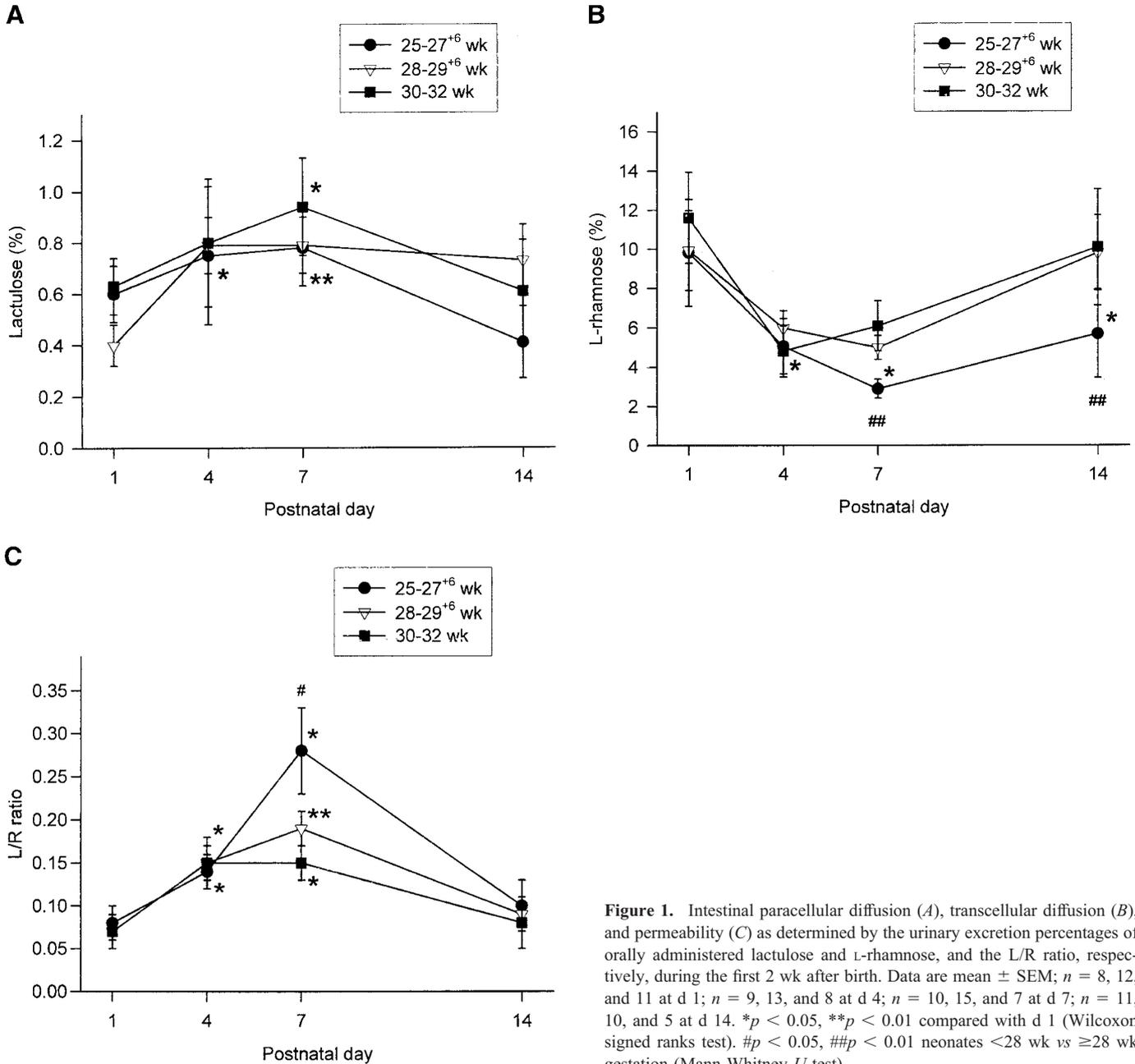


Figure 1. Intestinal paracellular diffusion (A), transcellular diffusion (B), and permeability (C) as determined by the urinary excretion percentages of orally administered lactulose and L-rhamnose, and the L/R ratio, respectively, during the first 2 wk after birth. Data are mean \pm SEM; $n = 8, 12,$ and 11 at d 1; $n = 9, 13,$ and 8 at d 4; $n = 10, 15,$ and 7 at d 7; $n = 11, 10,$ and 5 at d 14. * $p < 0.05,$ ** $p < 0.01$ compared with d 1 (Wilcoxon signed ranks test). # $p < 0.05,$ ## $p < 0.01$ neonates < 28 wk vs ≥ 28 wk gestation (Mann-Whitney U test).

The pattern of urinary D-lactate excretion closely resembled that of the L/R ratio (Fig. 2). Because no significant differences in the D-lactate-to-creatinine ratio were noted among the groups, data obtained in the three gestational age groups were pooled at each time point. The D-lactate-to-creatinine ratio was significantly higher at d 7 as compared with d 1 (102 ± 21.5 versus $48 \pm 10.3, p = 0.001$), and subsequently decreased at d 14 after birth (60 ± 13.8).

Repeated measurements analysis demonstrated an increase in the urinary excretion percentages of xylose and 3-O-methyl-D-glucose for the group of neonates as a whole ($p = 0.007$ and $p = 0.028$, respectively). However, subgroup analysis showed that xylose excretion in group A did not change during the first 2 wk, and was significantly lower as compared with the other two groups at d 4 ($p = 0.024$) and 14 ($p = 0.002$) after birth

(Fig. 3A). In contrast, in group B, xylose excretion increased significantly at d 4 ($p = 0.011$), 7 ($p = 0.005$), and 14 ($p = 0.036$) as compared with d 1. Xylose excretion was highest in group C, and remained at a constant level during the study period.

The changes in the urinary excretion percentages of methylglucose paralleled those of xylose. In group A, methylglucose excretion did not significantly increase during the 2 wk, and was significantly lower as compared with the other two groups at d 4 ($p = 0.043$) and 14 ($p = 0.003$) after birth (Fig. 3B). In group B, a significant increase in methylglucose excretion was observed at d 4 ($p = 0.041$), 7 ($p = 0.016$), and 14 ($p = 0.017$) as compared with d 1. Urinary excretion of methylglucose at d 1 after birth was highest in group C, and remained at this level during the first 14 d after birth.

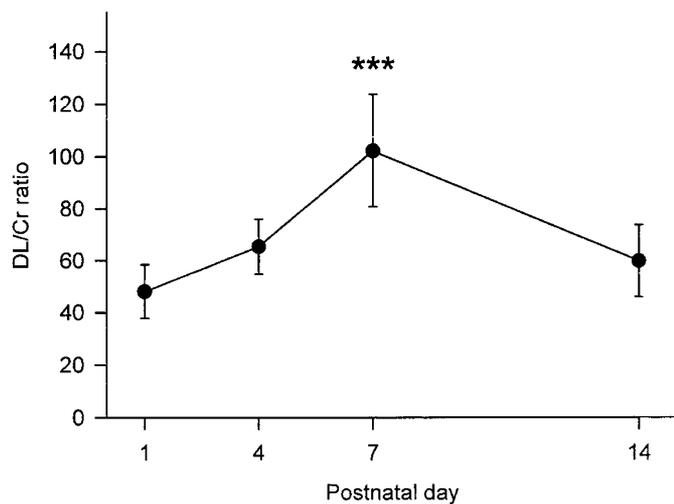


Figure 2. Urinary D-lactate excretion during the first 2 wk after birth in neonates of 25–32 wk gestation; D-lactate concentration is expressed relative to the creatinine concentration in the urine samples (*DL/Cr ratio*). Data are mean \pm SEM; $n = 45, 46, 47,$ and 38 at d 1, 4, 7, and 14, respectively. *** $p < 0.001$ compared with d 1 (Wilcoxon signed ranks test).

We also evaluated the influence of the clinical variables mentioned in Table 1 on the urinary excretion percentages of each of the four saccharides and on the L/R ratio. We compared the neonates exposed to the particular variable with those who were not exposed. Comparisons were made for the group of neonates as a whole, as the numbers in the individual gestational age groups were too low to allow reliable statistical analysis. Only the L/R ratio at d 1 after birth was significantly higher in neonates whose mothers received steroids antenatally with those who did not (0.079 ± 0.01 versus 0.039 ± 0.01 , $p = 0.023$). This difference was not continued at later time points. No statistically significant differences were detected with regard to the other factors or the type of enteral feeding.

DISCUSSION

The neonate depends on its intestine for protection against microorganisms and for absorption of ingested nutrients. To date, little is known about the development of the barrier and nutritive functions of the intestine in preterm neonates. In the present study, we investigated the changes in intestinal epithelial permeability and carrier-mediated absorption of monosaccharides in preterm neonates born between 25 and 32 wk gestation. The data showed that intestinal permeability increased during the first week after birth, most markedly in neonates <28 wk, and subsequently decreased during the second postnatal week after the initiation of enteral feeding. Both passive and active carrier-mediated monosaccharide absorption at d 14 after birth were higher in neonates born between 28 and 32 wk as compared with those <28 wk gestation.

Previous studies provide conflicting data with regard to neonatal intestinal permeability. Both decreases and increases in permeability during the first month after birth have been reported (11–13). Interpretation of the findings of these studies is further hampered by discrepancies in gestational age, clinical

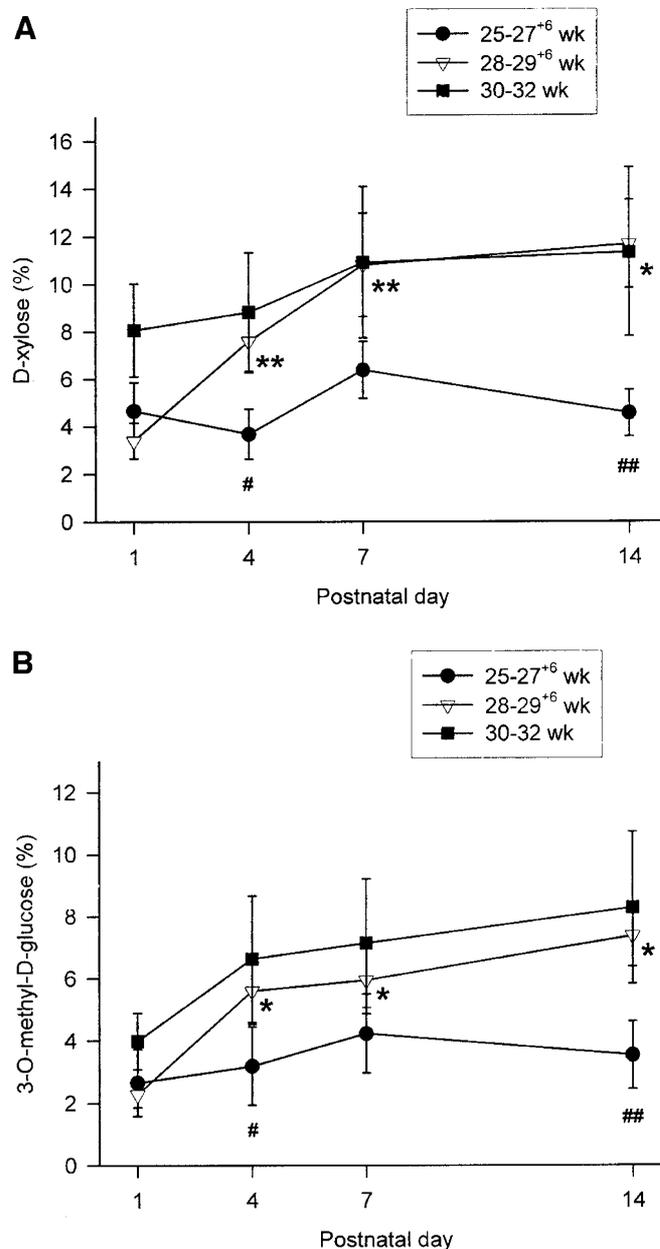


Figure 3. Intestinal passive (A) and active (B) carrier-mediated monosaccharide absorption as determined by the urinary excretion percentages of orally administered D-xylose and 3-O-methyl-D-glucose during the first 2 wk after birth. Data are mean \pm SEM; $n = 10, 13,$ and 11 at d 1; $n = 11, 13,$ and 8 at d 4; $n = 10, 15,$ and 8 at d 7; $n = 11, 10,$ and 5 at d 14. * $p < 0.05$, ** $p < 0.01$ compared with d 1 (Wilcoxon signed ranks test). # $p < 0.05$, ## $p < 0.01$ neonates <28 wk vs ≥ 28 wk gestation (Mann-Whitney U test).

condition, feeding regimen, and postnatal age at study. We assessed the intestinal permeability at 1, 4, 7, and 14 d after birth in preterm neonates by determining the urinary L/R ratio. The L/R ratio at d 1 was similar in all neonates born between 25 and 32 wk gestation. Although there are unfortunately no data available on intestinal permeability in older neonates using a similar L/R test, the L/R ratio in the current study was within the normal range previously reported for healthy infants and adults (18, 21). Together this may suggest that a mature level of intestinal epithelial barrier function is already attained by 25 wk gestation.

The increase in L/R ratio during the first week after birth was in part because of a decrease in rhamnose excretion in all neonates, most markedly in those born between 25 and 28 wk gestation. Because rhamnose crosses the intestinal epithelium predominantly via passive diffusion through small aqueous pores in the cell membranes of villus epithelial cells (9, 10), these data indicate that transcellular diffusion across the intestinal epithelium decreased during the first week after birth. In neonates ≥ 28 wk gestation, the increase in L/R ratio also resulted from an increase in lactulose excretion. Because lactulose permeates the intestinal mucosa via paracellular diffusion through the tight junction complexes between adjacent epithelial cells (9, 10), these data indicate that the leakiness of the tight junctions had increased at d 7 after birth in these neonates.

Interestingly, the L/R ratio declined after the initiation of enteral feeding during the second week of life. This suggests that the increase in intestinal permeability during the first postnatal week was at least partly related to the absence of nutrients in the intestinal lumen. Similar increases in permeability have been found in adult patients for whom total parenteral nutrition is the sole nutritional source (22). Furthermore, it has been demonstrated that enteral starvation of a previously normal gut is accompanied by jejunal mucosal hypoplasia (23). A reduction of intestinal villous height may lead to a diminished mucosal surface area for the diffusion of rhamnose, while at the same time facilitating the diffusion of lactulose in the crypt regions (24). In addition, other factors, such as the release of cytokines and intestinal hypoxia during periods of intestinal hypoperfusion, may contribute to the increase in intestinal permeability by attenuating epithelial tight junction functioning (25, 26).

The clinical significance of an increase in intestinal permeability for sugar probes is still a subject of investigation. Although the assumption has been made that alterations in gut barrier function as assessed by changes in intestinal permeability predispose to bacterial translocation and septic complications, there is yet no evidence in humans to support this view (27, 28). A direct relation between increased permeability and enhanced passage of luminal factors has thus far only been demonstrated for IgA immune complexes, ovalbumin, and bacterial chemotactic peptides (29–31).

The rise in L/R ratio between d 1 and 7 was accompanied by an increase in urinary D-lactate excretion. D-Lactate is derived from the fermentation of unabsorbed carbohydrates by bacteria present in the gut lumen (19). Therefore, urinary D-lactate excretion may increase as a consequence of enhanced production or increased permeation of the substance across the intestinal epithelium. Because total bacterial counts in the neonatal gut continue to increase during the first month after birth (32), it is unlikely that the transient peak at d 7 was related to increased bacterial production. The increase in D-lactate excretion therefore most likely resulted from increased intestinal uptake, thereby supporting the hypothesis that increased intestinal permeability enhances the transmucosal passage of substances from the gut lumen.

In addition to the permeability of the intestinal epithelium, we also assessed the early postnatal development of passive

and active carrier-mediated monosaccharide absorption. To this end, we measured the urinary excretion percentages of D-xylose and 3-O-methyl-D-glucose, respectively, the absorption of which mainly occurs in the proximal small intestine (33, 34). Interestingly, in neonates born between 28 and 30 wk, absorption of these monosaccharides rapidly increased to the levels of the older neonates at the end of the first postnatal week, even in the absence of enteral nutrients. In contrast, in neonates born between 25 and 28 wk, the absorption of xylose and methylglucose remained considerably lower throughout the first 2 wk after birth. These changes in intestinal uptake of xylose and methylglucose with time could not be explained by alterations in pre- or postmucosal factors, including gastric emptying, intestinal transit, or renal clearance, as this would have affected the excretion of these two monosaccharides and rhamnose equally. Taken together, our data indicate that the capacity of the gut to absorb monosaccharides by passive as well as active carrier-mediated transport mechanisms is very limited in neonates born at < 28 wk gestation throughout the early postnatal period.

To our knowledge, this is the first study that provides insight into the early postnatal development of the barrier function of the intestinal epithelium as well as of the sugar absorptive capacity in preterm neonates. Our data indicate that premature exposure of the immature intestine to the extrauterine environment in the absence of enteral nutrients is associated with a reduction in epithelial integrity. Both a diminished barrier function and a low absorptive capacity during the early postnatal period, particularly in neonates born at < 28 wk gestation, may underlie the high vulnerability of these patients to intestinal complications. Given the finding that epithelial integrity was restored on initiation of enteral feeding, early administration of enteral nutrition may offer an effective strategy to support the intestinal adaptation to extrauterine life in preterm neonates.

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