Delayed Disaccharidase Development in a Rabbit Model of Intrauterine Growth Retardation

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ABSTRACT

Intrauterine growth retardation (IUGR) affects almost 10% of infants born in the United States. It may be responsible for delayed gastrointestinal function and is an important cause of perinatal morbidity and mortality. The New Zealand White rabbit provides an optimal model for the study of naturally occurring IUGR. At term, birth weight is determined by fetal position within the bicornuate uterus. The small intestinal disaccharidase enzymes are indicators of bowel maturity and function. To examine potential differences in disaccharidase development between normal and IUGR fetuses, this rabbit model was investigated. Jejunum was harvested at multiple stages in rabbit development including the third trimester fetus, neonate, and adult. Lactase, maltase, and sucrase enzyme activity, as well as total protein content, was determined. Results were analyzed by the

2-tailed *t* test and ANOVA. Lactase activity appeared in the mid-third trimester, peaked in the early neonatal period, then declined to adult levels. Maltase activity appeared in the early third trimester and gradually rose to adult levels. Sucrase remained at trace levels until the mid-neonatal period, reaching adult levels by weaning. Both lactase and maltase activity were depressed in IUGR fetuses compared with their normal littermates. This pattern of disaccharidase depression continued into the neonatal period until catch-up growth occurred at 2 wk when levels equalized. This report describes differential small intestinal disaccharidase development between normal and growth-retarded rabbit fetuses in a naturally occurring model of IUGR. (*Pediatr Res* 50: 520–524, 2001)

The third trimester is a critical period in the maturation of the fetal gut for the postnatal functions of nutrient propulsion and absorption. The terminal stages of digestion are accomplished by the disaccharide hydrolases, disaccharidases, which are located on the enterocyte brush-border membrane. Disaccharidase activity parallels the digestive needs of the animal and is indicative of intestinal function and maturity. Disaccharidase ontogeny has been described in human (1) and animal models, including fetal and neonatal rabbits (2). Developmental delays in fetal disaccharidase activity have been reported in rat models of surgically created intrauterine growth retardation (IUGR) (3) and fetal rabbit gastroschisis (our unpublished observation).

The pregnant New Zealand White rabbit has a bicornuate uterus containing multiple fetuses within individual amniotic cavities (Fig. 1). By convention, the fetus at the ovarian end is numbered 1. At term, the weight ratio of the growth-retarded

fetus (position 3) to the ipsilateral normal fetus (position 1) is consistently 0.85, an example of naturally occurring IUGR (4). We hypothesize that IUGR rabbits are not only small but also have immature gastrointestinal development that impairs postnatal bowel function. To test this hypothesis, we studied the development of small intestinal disaccharidase enzymes. These enzymes are indicators of intestinal maturity and function in both normal and naturally occurring rabbit IUGR.

METHODS

All procedures were performed in accordance with the UCLA animal care policy and with approval of the Animal Use Committee of the institution. Twenty-six pregnant New Zealand White rabbits between gestational d 17–19 of their normal 31-d gestation and weighing 3.4 to 4.1 kg were obtained from a commercial vendor. Rabbits were housed in separate cages with 12-h light/12-h dark cycles, allowed free access to water and standard chow, and acclimated to their surroundings. For the neonatal studies, the litters from eight does delivered spontaneously, with the day of delivery recorded as neonatal d 1. Neonates were exclusively nursed by their mother.

Fetuses were studied on odd-numbered gestational days throughout the third trimester until term (d 21–31). The ma-

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SCHEMATIC OF RABBIT BICORNUATE UTERUS

Figure 1. Schematic of the rabbit bicornuate uterus. By convention, numbering commences at the ovarian end. The favored or normal fetus is in position 1; the runt or IUGR fetus is in position 3.

teral doe was killed using a lethal dose of i.v. Nembutal (100 mg/kg) on the appropriate gestational day. The uterus was immediately exposed through a midline laparotomy, and fetuses were removed from the favored and runt positions from each uterine horn, yielding four fetuses. After fetal weight was recorded, each was killed by cervical dislocation. The small intestine was removed, and a segment of midjejunum was immediately frozen on dry ice. Specimens were stored at -70°C for later analysis.

Neonatal rabbits were studied every fifth day from delivery through the first month of life. They were killed by cervical dislocation and the small intestine harvested as described above. Finally, the midjejunum of each maternal rabbit as well as six nonpregnant adult female rabbits was sampled as adult controls.

Jejunal samples were assayed for the disaccharidase enzymes lactase, maltase, and sucrase according to the method of Dahlqvist (5). All enzyme determinations were made under conditions of linear activity with respect to time and enzyme concentration. Because sample size and tissue fragility did not permit removal of the mucosa in the fetuses, all tissues were analyzed using full-thickness jejunum. Standard commercial laboratory-grade ($>99\%$ pure) reagents were used. Substrates were 100-mM solutions of the corresponding disaccharide. Enzymes were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Spectrophotometer readings were performed on a Beckman DU-20 (Beckman Coulter, Inc., Fullerton, CA, U.S.A.) after calibration with a known glucose standard. All assays were run in triplicate with a coefficient of variation $<7\%$. Protein content was determined using the Bio-Rad reagent kit (Sigma Chemical, St. Louis, MO, U.S.A.) with standard curve calculation using crystallized BSA ($>99\%$ pure).

Enzyme activity is reported as units enzyme (UE) per gram protein. One UE is defined as one micromole of substrate hydrolyzed per minute at 37°C under optimal conditions. Results are expressed as mean $\pm$ SEM. Statistical comparisons

were made between favored and runt fetuses and neonates by the 2-tailed paired t test and between various gestational and neonatal days by ANOVA. A p value < 0.05 was considered significant.

RESULTS

There was no fetal, neonatal, or maternal morbidity or mortality. Normal *versus* IUGR fetal and neonatal weights are shown in Figure 2. The runt showed progressive growth retardation throughout the third trimester compared with its normal-growth littermate. The weight ratio of the runt to the normal fetus was exactly 0.85 at term, as predicted from previous work in this model.

Jejunal protein content (mg/mL homogenate) increased throughout gestation but did not reach significance between time points or between the IUGR and favored fetuses (data not shown).

Disaccharidase activity is shown in Figure 3. Lactase appeared in the early third trimester, peaked in the early neonatal period, and subsequently declined to adult levels. Maltase activity appeared at the beginning of the third trimester, gradually rising to adult levels. Sucrase remained at trace levels throughout the entire fetal and early neonatal periods with an accelerated increase to adult levels by the time of weaning.

The raw data for lactase and maltase activity for IUGR fetuses and neonates compared with their normal littermates are shown in Tables 1 and 2. Figures 4 and 5 demonstrate this data graphically. Disaccharidase activity was lower in the growth-retarded fetuses at all gestational points. The decreased lactase and maltase enzyme activity continued into the neonatal period in those rabbits with lower somatic weights compared with their littermates until catch-up growth occurred by weaning.

DISCUSSION

Human IUGR, defined as term birth weight <2500 g, affects 3 to 10% of pregnancies in the United States and Canada (6). Afflicted infants have substantially increased morbidity and mortality compared with term infants of normal birth weight. The high cost to families and health care systems is docu-

Figure 2. Fetal weight in grams for normal and IUGR fetuses and neonates. Error bars are eliminated for clarity (SEM $< 1\%$). Significant differences were demonstrated between gestational d 25–31 ($p < 0.05$). The weight ratio of the IUGR to normal fetus is 0.85 at term.

Figure 3. Disaccharidase activity, fetuses and neonates. Error bars are eliminated for clarity (SEM < 1%). Lactase activity peaks in the neonatal period; maltase and sucrase gradually rise to adult levels after the neonatal period.

mented (7). It is estimated that two thirds of IUGR result from uteroplacental insufficiency for which there is no currently available prenatal treatment. IUGR models based on maternal caloric restriction or surgical manipulation have inconsistent fetal outcomes. Therefore, intrauterine therapeutic interventions have been difficult to evaluate (3, 8).

The New Zealand White rabbit provides a consistent model of naturally occurring IUGR and parallels human IUGR in several ways. The fetal rabbit runt achieves only 85% of the birth weight of its normal littermate, a decrement similar to the 10 to 15% birth-weight reduction in human IUGR. Although the cause of IUGR remains speculative, the runt rabbit fetus is housed at the watershed region between the uterine and vaginal arterial blood supply (4). This anatomic naturally occurring area of impaired blood supply is analogous to human uteroplacental insufficiency, a condition implying impaired total oxygen delivery. Finally, both rabbit and human IUGR occur early in the third trimester, and brain sparing is characteristic of each.

It has been postulated that *in utero* nutrient or hormone delivery may ameliorate IUGR, presumably by bypassing the defective placenta to deliver substrates to the fetus by direct infusion into the gastrointestinal tract or via ingested amniotic fluid (9). Radiolabeling studies in the fetal rabbit show that ingested nutrients are transported the length of the intestine and incorporated into growing somatic tissues (10). Rabbit and sheep fetuses increase their somatic weight and length after continuous amniotic infusion of nutrients (8, 9, 11). We hypothesize that the rabbit runt was not just smaller than its littermate but physiologically impaired by immature development of the gastrointestinal system.

The ontogeny of gastrointestinal development has been extensively studied, primarily in rat and mouse models. Transition periods, such as the timing of postnatal weaning, and the role of hormonal manipulations with corticosteroids and thyroxine are of particular interest. The reader is referred to a review by Henning (12), as a full summary is beyond the scope

of this report. The current study focuses on prenatal gastrointestinal development to complement the vast information available on postnatal animals.

The developing enterocyte is particularly susceptible to hypoxia (13, 14). There is no gross difference in enterocyte histology, including the height and width of the villi, in the rabbit runt *versus* its normal littermate (Phillips JD, personal communication). Disaccharidase enzyme activity is an indicator of small intestinal maturity and function in human neonates. Disaccharidase enzyme development in rabbits has been previously reported by Toofanian (2). However, results were pooled from entire litters and did not account for possible differences between individual fetuses based on uterine position and fetal size as in the current report.

Lactase, a β -disaccharidase, appears early in the third trimester and peaks at birth as breast milk becomes the primary nutrient in the neonate mammal. Levels then decline to adult values (2, 8, 15). Fetal human lactase development is similar, with negligible levels between gestational wk 10–26 that gradually increase until wk 38 when neonatal levels are attained. A decrease to adult levels occurs in childhood (15) as the diet becomes less dependent on lactose intake. Our study confirms this overall pattern of lactase activity in the rabbit.

When analyzed by fetal position, lactase levels were equivalent when body weights were similar early in the third trimester. However, as gestation advanced and growth retardation became evident, lactase levels were significantly lower in the IUGR fetus. IUGR lactase deficiency was prenatally determined and persisted into the neonatal period until catch-up growth was achieved.

The developmental pattern differs for the α -disaccharidases, which include sucrase and maltase. Sucrase remains at trace or low levels during the fetal period whereas a marked rise to adult levels commences at birth, perhaps preparing the animal for weaning (2). This pattern was confirmed. Only trace sucrase levels were detectable, and there was no difference between normal and IUGR fetuses. Maltase levels rise earlier during the third trimester. Similar to lactase, a significant reduction in maltase activity was present in the IUGR fetus during late gestation.

The runt neonatal rabbits had reduced lactase and maltase activity compared with their larger littermates. Neonatal weights equalized from d 10 forward as the runts exhibited catch-up growth; lactase and maltase activity was similar between these neonates. Although likely, it cannot be verified from the current study design that the small neonates were from the runt position *in utero*.

This observation of not only somatic but also physiologic impairment in IUGR fetuses supports further study into the underlying mechanisms governing these differences. Planned areas of investigation include the transcriptional and posttranscriptional regulatory events effecting developmental processes (*e.g.* lactase phlorizin hydrolase (LPH) and maltase glycoamylase (MGA), *in utero* hormonal manipulation to effect precocious maturation, and postnatal dietary and/or hormonal manipulation to expedite recovery from IUGR.

Table 1. Lactase activity, UE/mg

	Favored fetus			Runt fetus			<i>p</i> value
	N	Mean	SD	N	Mean	SD	
GD 21	4	Trace	N/A	4	Trace	N/A	NS
GD 23	6	0.6	0.9	6	Trace	N/A	0.12
GD 25	6	10.5	3.6	6	7.7	2.1	0.02**
GD 27	7	16.9	4.1	7	13.9	5.9	0.02**
GD 29	6	26.2	4.1	6	19.5	4.7	0.03**
GD 31	6	27.9	5.0	6	23.5	8.0	0.05**
ND 1	4	34.4	15.9	3	22.1	4.6	0.004**
ND 5	5	42.7	15.0	5	38.7	2.3	NS

N indicates number of animals; GD, gestational day; ND, neonatal day; NS, not significant.

** Statistical significance, *t* test.

Table 2. Maltase activity, UE/mg tissue

	Favored fetus			Runt fetus			<i>p</i> value
	N	Mean	SD	N	Mean	SD	
GD 21	4	Trace	N/A	4	Trace	N/A	NS
GD 23	6	3.9	1.5	6	2.5	1.5	NS
GD 25	6	8.3	3.5	6	6.4	2.8	0.02**
GD 27	7	6.4	2.5	7	3.5	2.4	0.04**
GD 29	6	7.9	0.9	6	7.3	1.0	0.NS
GD 31	6	9.0	1.5	6	7.1	1.4	0.04**
ND 1	4	7.9	4.0	3	5.3	2.6	0.02**
ND 5	5	16.6	6.6	5	10.3	1.2	0.05**

N indicates number of animals; GD, gestational day; ND, neonatal day; NS, not significant.

** Statistical significance, *t* test.

LACTASE ACTIVITY

Figure 4. Lactase activity (UE/g protein) in normal and IUGR fetuses and neonates. Significant differences manifest on gestational d 25–31 ($p < 0.05$).

MALTASE ACTIVITY

Figure 5. Maltase activity (UE/g protein) in normal and IUGR fetuses and neonates. Significant differences manifest on gestational d 25, 27, and 31 ($p < 0.05$).

SUMMARY

This current study demonstrates the reliability of the rabbit model for the study of naturally occurring IUGR. The IUGR rabbit fetus manifests delayed development of the disaccharidase enzymes lactase and maltase, which parallels somatic growth differences. They were enzyme deficient at birth, and the difference persisted until normal neonatal growth was achieved. Attempts to mature or normalize the functional capacity of immature fetal intestine, thereby preparing the host for adaptation to postnatal life, seem warranted.

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