

Alterations in Postnatal Intestinal Function during Chronic Hypoxemia¹

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ABSTRACT. Growth failure is a major complication of chronic hypoxemia, as seen in infants and children with cyanotic congenital heart disease. To determine whether chronic hypoxemia during infancy affects the gastrointestinal tract, we examined small intestinal growth and digestive enzyme activities in chronically hypoxemic newborn lambs and in age-matched controls. Chronic hypoxemia was produced by placing an inflatable occluder around the main pulmonary artery and performing a balloon atrial septostomy. Aortic oxygen saturation was reduced to 60–74% for 2 wk, after which the small intestine was removed for analysis. During chronic hypoxemia, somatic growth rate was decreased to 60% of control (hypoxemic, 135 ± 20 versus control, 216 ± 26 g/d, $p < 0.02$). No differences in caloric intake were found (hypoxemic, 129 ± 4 versus control, 128 ± 4 kcal/kg/d). Chronic hypoxemia did not alter small intestinal growth, as measured by jejuno-ileal weight, jejuno-ileal length, mucosal weight, or mucosal protein or DNA contents. However, sp act of lactase, the principal disaccharidase of the infant lamb intestine, were significantly decreased (hypoxemic, 0.08 ± 0.01 versus control, 0.146 ± 0.03 units of enzyme activity/mg DNA, $p < 0.05$), as were the total small intestinal contents of lactase (hypoxemic, 61.7 ± 7.0 versus control, 120.6 ± 21.7 units of enzyme activity, $p < 0.01$). There also were decreases in specific and total activities of other digestive enzymes such as maltase, amino-oligopeptidase, and alkaline phosphatase in hypoxemic intestine that did not achieve statistical significance. In conclusion, chronic hypoxemia in infancy is associated with decreases in activities of intestinal lactase, which may result in diminished capacity for absorption of dietary carbohydrate. Alterations in intestinal function may contribute to growth failure associated with chronic hypoxemia. (*Pediatr Res* 31: 234–238, 1992)

Abbreviations

AOP, amino-oligopeptidase
UE, unit of enzyme activity
VO₂, oxygen consumption

Growth failure is a major complication of chronic hypoxemia, as seen in infants and children with cyanotic congenital heart disease (1, 2). Although there have been several previous investigations to determine the etiology of this growth failure (3–7), the exact mechanisms are still not well defined. Several studies suggest that gastrointestinal tract function may be impaired during hypoxemia (3, 8–12); however, many of these experiments have been performed during acute rather than chronic hypoxemia (8, 9, 11, 12) and on adult rather than on immature subjects (8, 11, 12). Previous studies performed on hypoxemic human neonates were inadequately controlled for factors other than hypoxemia, such as prematurity, pulmonary disease, and heart failure (5, 13). Finally, data from previous studies using models of alveolar hypoxemia may not be applicable to patients with cyanotic heart disease, in whom hypoxemia is associated with intracardiac right to left shunting. In these patients, hemodynamic alterations secondary to structural heart defects may also contribute to the failure to grow.

To better elucidate the hemodynamic mechanisms underlying growth failure during chronic hypoxemia, Teitel *et al.* (6) developed a model of cyanotic congenital heart disease in the newborn lamb. These authors determined that chronically hypoxemic lambs grew at one fourth the rate of their age-matched controls. Using this model, we have previously reported a decrease in gastrointestinal tract blood flow and oxygen delivery during chronic hypoxemia, and have postulated that these changes could alter intestinal function (14). Thus, the purpose of the current study was to determine whether chronic hypoxemia, secondary to an intracardiac right to left shunt during the newborn period, results in alterations in growth or enzymology of the small intestine. Because it has also been suggested that reduced caloric intake may contribute to the growth failure associated with cyanotic congenital heart disease (3, 10), we also sought to determine whether chronic hypoxemia was associated with a decrease in dietary intake and, thus, in nutrient delivery to the gastrointestinal tract.

METHODS

Surgical preparation. Chronic hypoxemia was produced in 12 newborn lambs using a model of cyanotic congenital heart disease developed by Teitel *et al.* (6). Briefly, surgery was performed on newborn lambs of mixed Western breed during the 1st week of life. Polyvinyl catheters were inserted via a hind leg pedal artery and vein and advanced into the descending aorta and inferior vena cava. Under general anesthesia, a thoracotomy was performed in the 4th left intercostal space. Polyvinyl catheters were inserted into the ascending aorta, superior vena cava, right ventricle, pulmonary artery, and left atrium. A 5 F Fogarty dilation catheter (American Edwards Laboratories, Irvine, CA) was inserted via the hind leg pedal vein and advanced by direct visualization into the left atrium. A balloon atrial septostomy was then performed. The atrial septal defect was sized by passing the partially inflated balloon catheter across the defect, and additional septostomies were performed if necessary. Next, an

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inflatable silicone rubber balloon occluder with polyvinyl tubing was placed around the main pulmonary artery. This balloon occluder was left deflated during the immediate postoperative period and in this state was nonrestrictive. All catheters were filled with heparin, plugged, and brought to the skin via an s.c. tunnel and were protected by a zippered vest worn by the lamb. The lambs were then returned to their cages and were bottle-fed throughout the study period. The intravascular catheters were flushed with saline and re-heparinized daily during the 2-wk study period. Antibiotics (Dual-Pen; Tech America, Kansas City, MO) were given intramuscularly immediately before each catheter flushing. Intramuscular iron dextran complex (equivalent to 100 mg of elemental iron) was given weekly to avoid the hemodynamic effects of iron deficiency.

After the lambs recovered for 3 d, hypoxemia was produced by gradually inflating the pulmonary arterial occluder balloon with saline, which partially obstructed the right ventricular outflow tract and induced atrial right to left shunting. The details of this gradual inflation procedure have been published previously (6). Aortic oxygen saturation was measured by hemoximeter (OSM3; Radiometer, Copenhagen, Denmark). By adjusting the degree of balloon inflation, aortic oxygen saturation was decreased to 60 to 74% and was maintained at this level for 2 wk. Ten additional lambs also underwent thoracotomies and placement of intravascular catheters but did not have atrial septostomies or balloon occluders placed and served as normoxic controls.

The lambs were bottle-fed Land O'Lakes (Arden Hills, MN) lamb milk replacer, consisting of 39% carbohydrates (primarily lactose), 24% protein, and 30% fat. This formula supplies 0.9 kcal/mL. The volume and frequency of feedings were a maximum of 475 mL daily in six separate feedings from the 1st to the 3rd days of life, a maximum of 710 mL daily in three feedings up to 2 wk, and then *ad libitum* quantities in three daily feedings afterwards. Daily oral intake and weekly weight gain were recorded. Growth rate was calculated as weight gain (g/d).

Analysis of intestinal tissues. Investigations of intestinal tissues were performed on 12 lambs after 2 wk of hypoxemia [age 27 ± 4 (SD) d] and on 10 age-matched controls (age 24 ± 2 d, NS). All lambs were killed at least 2 h after their last feeding to minimize artifacts secondary to recent oral intake (15). After the lambs were killed with i.v. pentobarbital (90 mg/kg), the small bowel was rapidly removed from the pyloric sphincter to the cecum and the total length was measured after extension of the intestine to its full length by gentle traction. The intestine was rapidly divided into four equal segments, flushed with cold isotonic saline, dried, weighed, and placed over ice. Fifteen percent of total length of the proximal segment was taken from the midpoint and used for biochemical analysis. The segment was incised longitudinally over ice, and the mucosa was removed using a glass slide, weighed, and homogenized in nine volumes of 10 mM sodium phosphate, 5 mM Tris-HCl, 0.002% Triton X-100, pH 6.0. DNA and protein were quantitated by fluorometric assay (16) and by the method of Lowry (17), respectively. Lactase and maltase were measured by the glucose oxidase colorimetric assay of Dahlquist, as modified by Tsuboi (18). Cellobiose (15 mM) was used as substrate for measurement of brush border activities of lactase. Activity of AOP was determined by the method of Wojnarowska and Gray (19). Alkaline phosphatase activity was determined by the *p*-nitrophenylphosphate method (20). $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity was determined by the method of Yoshida as modified by Matsukawa (21). UE were expressed as μmol of substrate hydrolyzed per min. Specific activities were expressed as enzyme activity per mg of DNA. Total intestinal enzyme activities were calculated by multiplying the enzyme content of the analyzed segment by 6.66 to arrive at the total enzyme content of the segment analyzed, then multiplying by 4.

Statistical comparisons were made with an unpaired *t* test, and data are shown as the mean \pm SEM. Because we were comparing

only one experimental group with one control group under only one set of experimental conditions, we considered the individual *t* tests to be members of separate families, and, thus, we did not perform a Bonferroni correction for multiple comparisons (22, 23). Statistical significance was considered achieved when $p < 0.05$.

RESULTS

Somatic growth and dietary intake. Lambs in the experimental group grew normally until the onset of hypoxemia, after which they grew at approximately one third the rate of the controls (Fig. 1A). Caloric intake per kg of body weight per d in hypoxic lambs was not different from that in the controls at any time (Fig. 1B). Caloric intake averaged 129 ± 4 kcal/kg/d in hypoxic lambs versus 128 ± 4 kcal/kg/d in controls (NS). No significant changes were noted in total mL consumed per animal per d until 21 d of age, at which point growth had already slowed significantly. Therefore, chronic hypoxemia did not result in reduction of nutrient intake indexed to body size, indicating that alterations in somatic growth were not due to alterations in caloric consumption.

Intestinal growth and enzymology. There were no differences between the hypoxic and control groups in small intestinal weight or length (Table 1). Total mucosal weight, protein content, and DNA content were also similar in the two groups (Table 1). These data indicate that chronic hypoxemia, as produced by our model, did not result in impaired growth of the small intestine.

The sp act of lactase were diminished by 43% in hypoxic intestine ($p < 0.05$) (Fig. 2). This difference persisted when lactase activity was expressed per mg protein (hypoxic, 4.60 ± 0.42 versus control, 8.43 ± 1.85 UE/mg protein, $p < 0.05$). Specific activities of maltase and AOP were reduced, but failed to reach statistical significance. Although alkaline phosphatase ($p = 0.15$) and $\text{Na}^+\text{-K}^+\text{-ATPase}$ ($p = 0.29$) were decreased in the hypoxic lambs, the differences failed to reach statistical significance be-

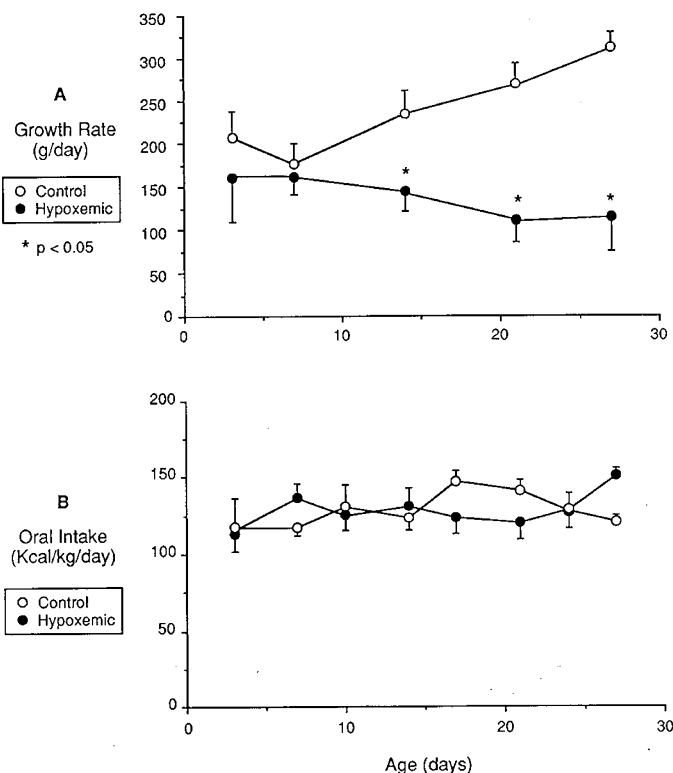


Fig. 1. Growth rate (A) and oral intake (B) indexed to total body weight compared between chronically hypoxic lambs and controls. *, $p < 0.05$ by *t* test.

Table 1. Comparison of selected intestinal parameters between hypoxemic and control lambs*

	Hypoxemic	Control
Total intestinal parameters		
Small intestinal wt (g)	164 ± 9	186 ± 15
Small intestinal length (m)	13.7 ± 0.4	13.3 ± 1.0
Mucosal parameters		
Total mucosal wt (g)	121 ± 8	139 ± 13
Mucosal protein content (g)	14.6 ± 1.1	16.6 ± 1.6
Mucosal DNA content (g)	0.89 ± 0.08	0.94 ± 0.08

* All were nonsignificant by *t* test.

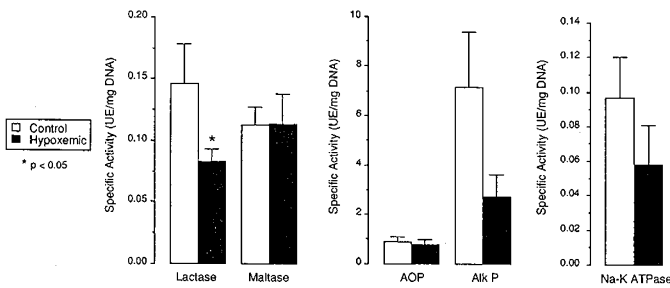


Fig. 2. Comparison of enzyme sp act, given as UE/mg DNA, between chronically hypoxemic and control lambs. *, $p < 0.05$ by *t* test.

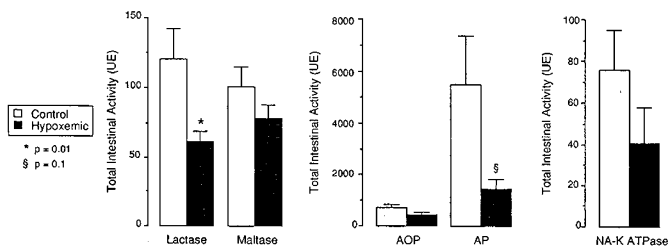


Fig. 3. Comparison of total intestinal enzyme activities, given as UE/intestine, between chronically hypoxemic and control lambs. *, $p < 0.01$; §, $p = 0.1$ by *t* test.

cause of large intragroup variance. Total intestinal activity of lactase was 49% lower in hypoxemic lamb intestine ($p = 0.01$) (Fig. 3). Total intestinal activities of maltase, AOP, alkaline phosphatase, and Na⁺-K⁺-ATPase were also lower in hypoxemic intestine, but these differences did not reach statistical significance.

DISCUSSION

In a model of cyanotic congenital heart disease in the newborn lamb, chronic hypoxemia did not result in impaired postnatal growth of the small intestine in spite of marked reduction in somatic growth. Small intestinal length, weight, total mucosal weight, and total mucosal DNA and protein did not differ between infant lambs that were hypoxemic for 2 wk and normoxemic controls (Table 1). This indicates that cellular proliferation in the small intestine of the infant lamb is not impaired by chronic hypoxemia. In contrast, the sp act (UE/mg DNA) of intestinal lactase, the major disaccharidase of the infant lamb intestine (1), were significantly lower in the hypoxemic animals, indicating that the cellular concentration of lactase was lower in the intestines of hypoxemic lambs (Fig. 2). The decreased total intestinal activity of lactase (Fig. 3) is therefore not due to diminished numbers of absorptive cells having normal complements of lactase, but to normal numbers of cells that have lower lactase activity per cell. It is known that lactase undergoes intracellular synthesis, processing, and transport to the brush border surface where degradation of the enzyme occurs (24, 25). Diminished catalytic activities of lactase in the current studies may be due to diminished synthesis of the enzyme (25), altered intracel-

ular processing resulting in either intracellular degradation (26) or inactive enzyme protein (27), or slowed transport and delivery to the brush border or may be a consequence of more rapid degradation after insertion into brush border sites (24). It is tempting to speculate that the mechanism that produces the reduction in catalytic activity is a mechanism that is directly linked to hypoxemia. Alternatively, we have previously described a decrease in gastrointestinal tract blood flow and oxygen delivery during chronic hypoxemia using this same model (14). Thus, the abnormalities of lactase in the current study could be secondary to the direct effects of reduced oxygen tension, to altered gastrointestinal tract blood flow, or to altered gastrointestinal tract oxygen delivery. In this regard, we have shown that reductions in intestinal oxygen delivery without commensurate decreases in blood flow are not associated with abnormalities of growth (28). We have also shown that chronically hypoxemic lambs do not manifest signs of right heart failure, which could interfere with intestinal function (6). However, further studies will be necessary to better separate the effects of hypoxemia from those of altered hemodynamics.

Although chronic hypoxemia resulted in cessation of somatic growth in our lambs (Fig. 1A), it is unlikely that the alterations observed in the small intestine are due to malnutrition. Nutrient consumption, expressed as the caloric intake per kg body weight, was not different between the two animal groups (Fig. 1B). Malnutrition produces characteristic changes in the small intestine that include mucosal atrophy [diminished mucosal protein and DNA and decreased rates of cellular proliferation (29)], as well as a characteristic array of enzymic alterations. Lactase activity levels are increased in malnutrition, whereas the activities of other digestive hydrolases, such as sucrase and maltase, are decreased (30, 31). In contrast, the small intestine of hypoxemic lambs showed no differences in mucosal weight, protein, or DNA contents. This indicates that the amount of absorptive surface is the same in the two animal groups, inasmuch as mucosal DNA and protein are highly correlated with villus height, villus number, and mitotic index (32). In addition, both specific and total activities of intestinal lactase were decreased, not increased, in chronically hypoxemic lambs, suggesting that this change was not due to malnutrition.

Analysis of total intestinal activities of the digestive hydrolases is useful because expressing the data as total activity removes the possibility of artifact when the data are related to DNA or protein (33) and total intestinal levels of digestive enzymes are correlated with the digestive capacity for the substrate of the enzyme. The decrease in total activities of lactase demonstrated in this study suggests that chronic hypoxemia during the newborn period may result in diminished digestive capacity for lactose, the major dietary carbohydrate consumed during infancy. This may result in diminished absorption of lactose and other nutrients, either producing or contributing to the profound growth failure seen in hypoxemic infant lambs. It is of interest that the hypoxemic lambs in our study did not show any of the clinical signs of lactose intolerance, such as diarrhea. This suggests that although hypoxemic lambs had diminished total intestinal capacities for digestion of lactose the levels of lactose consumed did not exceed that capacity.

Our finding of decreased lactase activity is in contrast to the results of previous studies by Lifshitz *et al.* (11) and Berant *et al.* (8) in adult animals, which failed to show changes in brush border disaccharidase levels after short intervals of alveolar hypoxemia. This is most likely attributable to the longer duration of hypoxemia in the current study, but might also reflect differences between species, the influence of additional hemodynamic abnormalities in our model that are not present during alveolar hypoxemia, or possibly a unique susceptibility of the infant intestine to the effects of hypoxemia. Previous studies of acute hypoxemia in adult animals have also demonstrated a reduction in absorption of the actively transported sugars glucose and galactose (8, 9, 12) and reduction in activity of the Na⁺-K⁺-

ATPase. In our studies, sp act of alkaline phosphatase were reduced by 72% and sp act of $\text{Na}^+\text{-K}^+\text{-ATPase}$ were reduced by 40% in intestines of chronically hypoxemic lambs compared with control. Although these results did not achieve statistical significance because of large variances, it is possible that these trends indicate that the activities of these two enzymes are indeed reduced in chronically hypoxemic infant intestine. Others have demonstrated alterations in glucose, protein, and fat absorption in hypoxemic human infants, although the effects of other factors such as prematurity and heart failure were not well controlled (5, 13). One study was performed using breath test techniques to evaluate lactose malabsorption in a group of 20 infants with cyanotic and acyanotic heart disease (34). Although only two infants were found to have positive breath tests, the breath test methodology was not validated for use in this clinical setting by the authors. In particular, a nonabsorbable carbohydrate, such as lactulose, should have been administered as a control to show that appreciable levels of hydrogen were excreted in breath. It is theoretically possible that in the presence of cyanotic heart disease, decreased blood flow to the lungs could result in impaired pulmonary excretion of hydrogen resulting in false negative results.

Past studies have produced conflicting results regarding the role of oral intake in hypoxemic growth failure. Elliott and Cheek (10) measured oral intake in hypoxemic rats and demonstrated that pair-fed normoxic rats also failed to grow, although the severity of the hypoxic growth failure was not totally accounted for by reduced intake (3). Studies in hypoxemic human infants (7, 35) and in children living at high altitude (36, 37) have also produced conflicting results as to the exact contribution of malnutrition in the etiology of hypoxic growth failure. Although several studies have described alterations in physical growth of infants and children living at high altitude (38, 39), interpretation of these studies has been confounded by differences in socioeconomic status and racial background (36, 37, 40). Finally, increased cardiorespiratory work during chronic hypoxemia may result in an increase in caloric requirements (4, 5). Although total body VO_2 in our lambs has been shown to be unchanged from control levels (6, 7), an increase in cardiorespiratory VO_2 may be counterbalanced by a decrease in VO_2 directed toward growth (6, 41).

A potential limitation of the current study concerns the appropriateness of the lamb as a model for the study of digestive enzyme function in cyanotic congenital heart disease. The advantages of this model are that the hemodynamic characteristics and failure of growth have already been well characterized (6, 14, 28). Developmental differences in digestive enzyme activity have been little studied in the lamb. Lactase and maltase activities have been characterized in the newborn lamb and have been shown to remain at stable levels during the first 3–5 wk of life (42). Generally, digestive enzymes exhibit an aboral gradient in most species, and this may be the case for lactase in the infant lamb intestine (43). Because of the likelihood that gradients were in fact present in our suckling lambs, we based our calculations for total intestinal lactase on lactase activities measured in the proximal jejunum, where they have been shown to be maximal in other mammalian species. On this basis, total intestinal lactase activities that we calculated represent the maximum hydrolyzing capacity of the small intestine. The actual total activity could have been lower. Although the sheep is a ruminant, representing a major difference in intestinal tract function from that in the human, rumen function normally does not begin until after 21 d of age and is also dependent on institution of grass feeding, so that ruminant function may be delayed with continued milk replacer feeding (42–44). The current study also does not attempt to isolate the effects of chronic hypoxemia from the additional hemodynamic, hematologic, and neurohumoral alterations present secondary to right ventricular outflow tract obstruction and atrial right to left shunting. In this regard, we have previously shown that arterial and central venous pressures are not altered

in chronically hypoxemic lambs (6). However, it is possible that an increased level of stress during the initial days of hypoxemia could have partially accounted for our results. Although we have not studied digestive function in lambs with longer periods of chronic hypoxemia, Dalinghaus *et al.* (Dalinghaus M, personal communication) have shown that growth failure persists in this model for at least 4 wk of hypoxemia. Finally, our model is also potentially limited in that it does not produce *in utero* hemodynamic alterations or hypoxemia immediately after birth, as would be present in many infants with cyanotic congenital heart disease. We acknowledge this limitation in our model, however, and recognize that there are many cyanotic congenital lesions that are not associated with severe hypoxemia at birth, in which progressive hypoxemia develops only over the first few months of life.

In conclusion, chronic hypoxemia during the newborn period, associated with an intracardiac right to left shunt, produced significant reductions in both the sp act and total activities of intestinal lactase. This may result in a major decrease in the capacity for digestion of lactose, the predominant dietary carbohydrate of the newborn period. Chronic hypoxemia does not result in significant alteration in dietary intake, which may account for the lack of differences in growth and mucosal cellularity of the small intestine, the portion of the gastrointestinal tract responsible for nutrient absorption. Developing a better understanding of the effects of hypoxemia on the gastrointestinal tract may allow for the development of altered nutritional regimens or enzyme replacement therapies that could ameliorate the effects of chronic hypoxemia on growth.

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