Restriction of Nutrition In Utero Selectively Inhibits Gastrointestinal Growth in Fetal Sheep1,2,3

Jeffrey F. Trahair,*4 Tania M. DeBarro,† Jeffrey S. Robinson‡ and Julie A. Owens**

Departments of *Anatomy & Histology, †Obstetrics & Gynecology, and **Physiology, University of Adelaide, South Australia, 5005, Australia

ABSTRACT This study examined the effects of reduced nutrition on fetal growth over the first half of gestation. Reduced nutrition was achieved by a combination of reduced maternal food intake and carunclectomy, a procedure which restricts the development of the placenta. There were no major effects of restriction on fetal body, tissue or organ growth, except for the gastrointestinal tract (GIT). Total GIT weight was lower in restricted fetuses than in controls. More specifically, it was growth of the small and large intestine which was compromised. Small intestinal weight was significantly lower, both in absolute terms and relative to body weight. The intestinal diameter and mucosal area were significantly lower in both small and large intestine of restricted fetuses. Maturation of enterocytes was also delayed in nutrient-restricted fetuses. In addition, there were focal lesions of the brush border present, indicating abnormal epithelial differentiation. By term, in growth-retarded fetuses, growth deficits in many organs were present, including the GIT. The present study suggests that GIT growth deficits may have a long-term etiology, including at their onset, abnormal cellular differentiation. These results could explain why GIT function in intrauterine growth-retarded infants is more likely to be compromised than in premature or term infants. J. Nutr. 127: 637–641, 1997.

KEY WORDS: • growth retardation • sheep • fetus • small intestine • development

Deficient or abnormal growth of the gastrointestinal tract (GIT) occurs when fetal body growth is compromised (Lebenthal et al. 1981, Shanklin and Cooke 1993, Shrader and Zeman 1969, Thornbury et al. 1993, Xu et al. 1994, Younaszai and Ranshaw 1973). In fetal sheep, long-term reduction in the supply of oxygen and/or nutrients clearly restricts fetal growth (Harding et al. 1985) and causes deficiencies in gastrointestinal growth, particularly of the small intestine (Avila et al. 1989). Intrauterine growth-retarded infants are at much greater risk of infection (Grenenwald 1963). Immune function, particularly mucosal immunity, is reduced (Watson and McMurray 1979) in low-birth-weight and poorly nourished infants. Gut function is compromised (Lebenthal and Leung 1988), and mucosally acquired infection is more prevalent in newborns and infants (Prindull and Ahmad 1993). In addition, although body growth might be restored by postnatal nutritional intervention, more commonly, suboptimal growth persists because of permanent changes in key GIT functions, such as epithelial permeability (Lunn et al. 1991).

The most rapid phase of GIT development in long gestation species such as sheep and humans is during the last trimester (Trahair et al. 1986a, Weaver et al. 1991). Previous studies have shown that in growth-restricted fetal sheep and pigs, defective GIT development, particularly of the mucosal tissues, is well established by late gestation (Avila et al. 1989, Xu et al. 1994), suggesting that the rapid growth phase has not been adequately matched with substrate delivery. This study tests the hypothesis that restriction of substrate supply in utero during the first half of gestation, as a result of restrained placental development and maternal undernutrition, alters the pattern of GIT growth early in development. Thus GIT deficiencies present at birth after compromised intrauterine growth might arise out of a long period of perturbed or abnormal growth which was initiated early in pregnancy. This information is vital in our understanding of the capacity for the neonatal GIT to undergo appropriate catch-up growth.

MATERIALS AND METHODS

All procedures involving animals were approved by the Animal Ethics Committee, University of Adelaide and complied with guidelines of the National Health and Medical Research Council of Australia.

Sheep were supplied by and housed at the Struan Research Centre, Naracoorte, SA (South Australian Department of Agriculture). Multiparous mature Border-Leicester × Merino ewes were randomly divided into two groups. For 2 mo prior to mating, the nutritional intake of each group was controlled by stocking rate to produce a well-fed (>60 kg for ewes of this genotype) and a restricted (15 kg below that of well-fed ewes) group. Live weight of the ewes was maintained at these levels throughout the mating period and up to

* Supported by grants from the National Health and Medical Research Council of Australia and the Channel Seven Children's Research Foundation of South Australia.
† Presented at the 10th World Congress of Gastroenterology, Los Angeles Conference Centre, October 2–7, 1994.
‡ The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.
§ To whom correspondence and reprint requests should be addressed.
* Abbreviations used: AEC, apical endocytic complex; GIT, gastrointestinal tract; IUGR, intrauterine growth retardation.
d 90 by pasture management (stocking rate) and supplementation with clover-grass hay (3 times/wk to the well-fed group). At d 90, the condition scores (an index of subcutaneous fat) (Russell et al. 1969) were assessed as an outcome measure of the feeding protocol.

Additionally, in the feed-restricted group, to further restrict substrate delivery to fetuses, nonpregnant ewes had ~85% of their endometrial implantation sites (caruncles) surgically excised from the uterus (a procedure known as carunclectomy). From the initial cohort of animals, some ewes failed to become pregnant or failed to maintain a pregnancy, whereas others carried singletons, twins or triplets. At the end of the study period, six (well-fed) and four (feed-restricted plus carunclectomy) surviving singleton fetuses were available for study.

At d 90 of gestation (term is ~147 d), the ewes were killed by a lethal injection of pentobarbitone (Lethabarb; Virbac, Peakhurst, Australia), followed by exsanguination. The ewe’s body and carcass weights were recorded. The fetus was quickly removed and body weight and length recorded, skeletal components measured, and GIT (and its component parts) and major organ weights recorded. Fetal skeletal components were also measured. Tissue samples from the GIT were dissected and fixed in Bouin’s fluid, for light microscopy, or 2.5% buffered glutaraldehyde for electron microscopy. Tissues were routinely processed for light (wax sections) and transmission electron (resin sections) microscopy. Proximal small intestinal samples were taken from within 5 cm of the ligament of Treitz, and distal small intestine and colon samples were within 5 cm proximal or distal to the ileo-cecal junction, respectively. The placenta was weighed and the cotyledons counted. Tissues were routinely processed for light (wax sections) and transmission electron (plastic sections) microscopy. Proximal small intestinal samples were taken from within 5 cm of the ligament of Treitz, and distal small intestine and colon samples were within 5 cm proximal or distal to the ileo-cecal junction, respectively. The placenta was weighed and the cotyledons counted. Tissues were routinely prepared for light (wax sections) and transmission electron (plastic sections) microscopy. From 3-µm wax sections, assessments of the intestinal diameter (at least 6 cross sections), villous height and crypt depth (10–15 were measured), villous or crypt density (villi or crypts/millimeter mucosal circumference) (at least 2 mm mucosal circumference assessed) were made and cross-sectional areas computed. Measurements were made using a camera lucida and digitizing tablet equipped with a diode cursor and computer software (Trace, Leading Edge, Adelaide, SA, Australia).

Means ± SEM are presented throughout. Comparison of means was by Student’s unpaired t test, and significance accepted at P < 0.05. Actual P values (as computed by the software Minitab, State College, PA) are given throughout.

RESULTS

At d 90, the condition scores of the ewes in the well-fed group (4.60 ± 0.24) were higher (P < 0.05) than those in the feed-restricted group (1.50 ± 0.20). Well-fed ewes maintained their mating body weight (65 ± 1 kg at mating; 65 ± 4 kg at d 90).

The placentas in the restricted group had significantly fewer cotyledons (40.5 ± 7.6, control; 75.2 ± 4.7, restricted, P = 0.003) and the total weight was lower (305 ± 54 g, control; 515 ± 38 g, restricted, P = 0.01).

Fetal weight in the restricted group tended to be lower than in the well-fed group (P = 0.11) (Table 1). Fetal crown-rump length was lower (P = 0.047). Of the major skeletal measurements made (head width and length, femur, humerus, tibia and radius length), only radius length was lower in the restricted fetuses (P = 0.048).

Absolute (P = 0.042) and relative (P = 0.036) GIT weights were reduced in the restricted group (Table 1). Small intestinal weight was significantly reduced by restriction, both in absolute terms (P = 0.018), and relative to body weight (P = 0.044). There were no significant differences in weight of any other region of the GIT as a result of reduced fetal nutrition (data not shown).

Duodenal, proximal and distal small intestinal, and large intestinal circumference were all significantly lower (−17, −10, −27, and −33%, respectively, P < 0.05) in the restricted group (Table 1). There were no significant differences in villus height (in the small intestine) or mucosal thickness (in the large intestine) between control and restricted fetuses (data not shown). Crypts were significantly smaller only in the duodenum and proximal small intestine (−11%, P < 0.05) of restricted, compared with control fetuses. The mucosal area in cross section was lower in all regions (−40, −34, −41, and −58%, respectively, as above, P < 0.05) in restricted, compared with controls.

Enterocyte differentiation was either retarded or abnormal in restricted fetuses. In fetuses of the well-fed ewes, the proximal small intestine had already developed an extensive network of vesicles and tubules in the apical cytoplasm. This network is known as the apical endocytic complex (AEC) (Fig. 1). In restricted fetuses, the AEC was sparse, or even absent from some cells (Fig. 2). The microvilli were shorter than in control fetuses, and large intracytoplasmic pools of coarse granular glycogen were present (Fig. 3). Although the above features are typical of the normal developing GIT, they are usually present only in much younger fetuses (Trahair and Robinson 1986). In addition, there were focal lesions of the brush border present, with groups of cells displaying apical cytoplasmic extensions (Fig. 4), or even an absence of microvilli altogether in enterocytes of restricted fetuses.

DISCUSSION

Placental restriction of fetal growth accounts for a substantial proportion of intrauterine growth retardation (IUGR) in sheep and other species, with fetal and placental growth becoming highly correlated in late gestation (Harding et al.

**TABLE 1**

Comparison of fetal growth in control and restricted fetuses

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight, g</td>
<td>732 ± 37</td>
<td>596 ± 73</td>
</tr>
<tr>
<td>Fetal crown rump length, mm</td>
<td>336 ± 11</td>
<td>291 ± 17*</td>
</tr>
<tr>
<td>Fetal radius length, mm</td>
<td>43 ± 2</td>
<td>35 ± 3*</td>
</tr>
<tr>
<td>Total GIT body weight, g</td>
<td>28.0 ± 1.9</td>
<td>18.1 ± 4.1*</td>
</tr>
<tr>
<td>GIT/body weight, g/100 g</td>
<td>3.9 ± 0.2</td>
<td>3.0 ± 0.3*</td>
</tr>
<tr>
<td>Small intestinal weight, g</td>
<td>17.3 ± 1.3</td>
<td>9.7 ± 2.5*</td>
</tr>
<tr>
<td>Small intestinal weight/body weight, g/100 g</td>
<td>2.4 ± 0.1</td>
<td>1.9 ± 0.2*</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM; *significant difference between control (n = 6) and restricted (n = 4) groups, P < 0.05, as determined by t test. 2 GIT, gastrointestinal tract.
was not affected, but gut growth, both in absolute and, importantly, in relative terms was markedly impaired. This was evident despite a wider variability in the somatic and placental growth measurements. The variability of these gross measures could have arisen via synergy between placental insufficiency and maternal undernutrition, possibly suggesting that if fetuses were examined at a later stage, a more pronounced somatic deficit, as would be expected from previous studies in late gestation fetuses, would have emerged.

In an earlier study in fetal sheep, our colleagues found that IUGR resulting from placental restriction reduced small intestinal length and weight by late term (136 d) (Avila et al. 1989). However, fetal weight was also reduced, and thus small intestinal weight relative to body weight was unaltered. Similar changes were observed in low-birth-weight newborn piglets (Xu et al. 1994). Only slight (P = 0.11) differences in body weight were evident by mid-gestation in the current study. We conclude therefore that somatic growth deficits must occur some time after the onset and establishment of the early phase of reduced gastrointestinal growth that we have identified in our study.

The substantial adverse effect of restriction in mid-gestation on the GIT is somewhat surprising because the most rapid phase of GIT growth in other long gestation species (humans) occurs during the last trimester, when the small intestine approximately doubles in length (Weaver et al. 1991). Consistent with this, we have demonstrated in the fetal sheep an accelerated rate of increase of most morphometric variables in late gestation (Trahair et al. 1986). In general, the gut grows more rapidly than does the body as a whole. After birth, this differential growth rate slows and the gut to body ratio declines (Weaver et al. 1991). In IUGR, although age and fetal weight correlate linearly with gut length, the relationships between these variables and gut weight are much more complex, suggesting that the composition of the gut wall is altered via different mechanisms (Shanklin and Cooke 1993). It is likely that different aspects of gut growth (for example, the various wall components) may be targets for altered growth at critical periods during ontogeny (Lebenthal and Lee 1988), and, furthermore, once altered growth has been initiated, the ontogenic processes and their regulation may also be specific. This suggests that unique tissue and body phenotypes arise out of altered growth patterns. The striking discovery that particular birth phenotypes correlated with the development of major diseases in later life such as cardiovascular disease and hypertension clearly demonstrates that some pathogenetic mechanisms may be established during life in utero (for an extended discussion, see Barker 1994). Whether this is the case for the GIT is not yet known.

As one functional and essential end point measure for successful development in utero, the GIT must possess an adequate surface area and capacity for digestion and absorption of nutrients when enteral feeding begins. In adults of many species this capacity is related to body size and metabolic rate (Chivers and Hladik 1980, Snipes and Kriete 1991), but it is also clear that considerable reserve exists. In contrast, it is apparent that in neonates this reserve capacity is absent or lacking because gut disease requiring resection can seriously jeopardize an infant’s chance of long-term survival and well being (Zeigler 1986). Currently, we do not know enough about the functional capacity of the immature GIT, particularly subsequent to restriction in utero, to manage these critical situations adequately.

Intestinal length is an important determinant of adequate intestinal performance. However, consideration of the complex geometry of the intestinal mucosa would a priori lead us
to conclude that the amplifying effects of altered growth of mucosal components on surface area, including changes in the density and size of the villi and microvilli, together with changes in diameter, are likely to be more important.

We have shown that although intestinal diameter undergoes ontogenic changes, a variety of perturbations of the intrauterine environment do not alter intestinal diameter, even when markedly altered growth has occurred in some wall components (e.g., villous height, crypt depth, villous and crypt density) (Trahair and Robinson 1987). Nethertheless, intestinal diameter remains highly correlated with the size of most of the GIT wall tissue components under a range of circumstances, demonstrating that even in altered growth there is considerable maintenance of balance of tissue components, and that even altered growth is an integrated process (Trahair and Robinson 1987). The factors which drive changes in diameter of the GIT are not known. Hypertrophy of GIT tissues can be brought about by distension, for instance, after intestinal obstruction (Touloukian and Wright 1973, Trahair et al. 1993), but absence of swallowing does not necessarily result in reduced diameter (Avila et al. 1989, Trahair and Harding 1992 and 1995, Trahair et al. 1986b).

A consistent finding in our many studies in fetal sheep is that the mucosal epithelium is the most labile wall component in the small intestine. This feature is retained as a major adaptive capacity in the adult. We have previously shown that enterocyte morphology is aberrant in fetuses in which swallowing has been ablated (Trahair and Harding 1992). While we have suggested that absence of putative gut growth factors in swallowed fluid may contribute to the abnormal ontogeny following absence of swallowing, luminal nutrition is also likely to be a contributory factor (Trahair 1993). In the present study, we were not able to assess major metabolites. However, elsewhere we have shown that by 120 d, carunclectomy-induced restriction of placental and fetal growth is characterized by reduced rates of oxygen and glucose delivery to the fetus and fetal hypoxia and hypoglycemia (Owens et al. 1994). Although reduced substrate delivery could impair organ growth, the preferred substrate for maintenance of enterocyte homeostasis is glutamine (Souba et al. 1990). Other studies in fetal sheep have shown that glutamine levels fall significantly in response to maternal food deprivation (Lemons et al. 1984). Because the caruncle is partially deprived in the present study were also undernourished, lowered glutamine levels could have contributed to reduced GIT growth, particularly at the cellular level.

The immaturity of the apical endocytic complex and reduced mucosal area would suggest that the growth-retarded fetal gut would have a reduced capacity to utilize substrates derived from fetal swallowing. If fetal hypoxia was present, it is possible that fetal swallowing was also depressed (Brace et al. 1994). Swallowed proteins present in amniotic fluid are hydrolyzed in the gut, within the enterocytes. In humans, it is estimated that 10–15% of daily whole-body protein deposition can be accounted for by the hydrolysis of material in swallowed fluid (Gitlin et al. 1972). This unique form of digestion relies on the development of intense endosomal capacity and lysosomal activity. The delayed and inadequate development of these uniquely fetal (and in some species, neonatal) GIT features could impair utilization of enteral substrates, further contributing to the major GIT growth failure which ensues later in IUGR. The presence of an abnormal or absent microvillus border in the small intestine of growth-retarded fetal sheep in mid-gestation confirms the findings of a previous study, in late gestation, which demonstrated a lack of a pericellular-Schiff’s reagent positive brush border at the light microscope level (Avila et al. 1989). This is consistent with the reduced barrier function, enhanced permeability, depressed biochemical markers (digestive enzymes) and impaired morphological maturation that have been noted in experimental and clinical growth retardation and reported elsewhere (Avila et al. 1989, Lebenthal et al. 1981, Shanklin and Cooke 1993, Shrader and Zeman 1969, Thornbury et al. 1993, Xu et al. 1994, Younasai and Ranshaw 1973).

The onset of GIT growth retardation occurs prior to major somatic growth deficits. The early appearance of altered pathways of cytokidifferentiation and reduced mucosal mass suggests that defective GIT function in growth-retarded infants arises as a consequence of long-term altered ontogeny of GIT development.

LITERATURE CITED


