Oral Insulin Increases Small Intestinal Mass and Disaccharidase Activity in the Newborn Miniature Pig

ROBERT J. SHULMAN

USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas 77030

ABSTRACT. Factors, such as insulin, found in human and pig colostrum and mature milk likely influence small intestinal growth and development. Although pharmacologic doses of insulin injected parenterally may accelerate small intestinal development in altricial animals such as the rodent, the effects of oral insulin on intestinal development have not been studied. In the first of two studies, we randomized 2-d-old miniature piglets to receive bottle-feedings of a swine weaning milk formula with (group F + I) or without (group F) the addition of insulin. Serum glucose, insulin, and cortisol were measured before and 1 h after the first feeding the piglets received at our facility. In the second study, piglets were randomized (groups F and F + I) and fed for 6 d, after which blood samples were obtained as in the first experiment. The piglets were then killed and the small intestine removed for analysis. We found no differences between groups in serum glucose, insulin, and cortisol at both 2 and 8 d of age, both before and after feeding. In the second experiment, small intestinal weight was greater in the F + I than in the F group. Although no differences were noted between groups in the jejunum, values were greater for group F + I versus group F for ileal mucosal weight, protein, RNA, lactase, and maltase activities. No differences were found between groups in ileal DNA or sucrase activity. We conclude that the administration of oral insulin stimulated an increase in ileal mass and disaccharidase activity in the newborn miniature pig without apparent concomitant changes in serum glucose, insulin, or cortisol. (*Pediatr Res* 28: 171-175, 1990)

decrease in the trophic activity of colostrum parallels the decrease in colostrum insulin concentration (1).

In previous animal studies of the effect of insulin on small intestinal growth and development, most investigators injected the insulin parenterally (8-11). Although humans and animals receive insulin enterally, e.g. in colostrum, previous investigators have not examined the effect of oral insulin on small intestinal growth and development. Our objective was to determine whether oral insulin alters the rate of small intestinal growth (length and mass) and development (disaccharidase activity). We studied the newborn miniature piglet, an animal whose level of gastrointestinal development more closely approximates that of the human newborn than does that of other commonly studied species such as the rat and mouse (12).

MATERIALS AND METHODS

Design. Hanford miniature piglet littermates (Charles River Breeding Laboratories, Wilmington, MA) were removed from the sow and shipped to our facility at 2 d of age. Piglets were weighed upon arrival and daily thereafter. Piglet littermates were randomized to receive a swine weaning milk-based formula (Sowena, Merrick's, Union City, WI) either with (group F + I) or without (group F) the addition of 85 mU/mL of regular porcine insulin (Regular Iletin II, Eli Lilly Co., Indianapolis, IN). In the first experiment, piglets received 20 mL of formula with or without insulin at the time of their arrival. Blood samples were obtained before and at 60 min after the first feeding. In the second experiment, the feeding regimen was continued for 6 d. On the 7th d of study, blood samples were obtained as in the first experiment. The piglets were then killed and the small intestine was removed for analysis.

Feeding. One L of formula was prepared daily for each group (with or without insulin). The respective formula was poured into bottles and nipple-fed at 0800, 1100, 1400, 1700, 2000, and 2400 h. Bottles were weighed before and after feeding and the intake calculated. The piglets were allowed to nurse (*i.e.* nipple) *ad libitum* except when the disparity in intakes among animals at each feeding was greater than 10%. In such cases, the piglets were pair-fed to maintain comparable intakes.

Tissue collection. Fentanyl (0.05 mg/kg) and ketamine (11 mg/kg), administered intramuscularly, were used to anesthetize the piglets. The abdomen was opened and the small intestine, from pylorus to cecum, was removed and placed in cold saline. Length was determined by suspending the small intestine and attaching a 35-g weight. The small intestine was flushed with cold saline and the mesentery was then stripped off. The duodenum was defined as the segment from pylorus to the ligament of Treitz (12, 13). The remaining small intestine was divided in half (*i.e.* jejunum and ileum) (12, 13). Each segment was weighed. Because of the large length of small intestine, the jejunum and ileum were divided into six equal segments (1 to 6, proximal to

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Correspondence and reprint requests: Robert J. Shulman, M.D., Children's Nutrition Research Center, 1100 Bates Street, Houston, TX 77030.

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distal). Jejunal segment 1 and ileal segment 3 were opened lengthwise and gently blotted. The mucosa was scraped off, and the mucosa and serosa were placed in preweighed tubes and frozen in liquid nitrogen.

Analyses. The blood samples were analyzed for serum glucose using an autoanalyzer (Cobas, Roche Diagnostic, Montclair, NJ) and for insulin and cortisol using a double-antibody RIA (Radioassay Systems Laboratories, Carson, CA). The mucosa was homogenized in deionized water and analyzed for protein by the method of Smith *et al.* (14). DNA was measured using the technique of Labarca and Paigen (15). RNA was determined using the modified Schmidt-Tannhauser procedure (16). Lactase, sucrase, and maltase activities were measured as previously described (12, 13).

Statistics. In the first experiment, 10 piglets (five litters of two littermates) were studied. In the second experiment, 24 piglets (six litters of four littermates each) were studied. Consequently, two-way analysis of variance was used with treatment group as one factor and litter as the other. Changes within individual animals were assessed using a paired *t* test. Data are expressed as mean $\pm$ SE.

RESULTS

In the first experiment, pre- and postprandial serum glucose values were similar between groups (Table 1). Serum glucose increased in both groups postprandially. Serum insulin values also were similar between groups before and after the feeding. Serum insulin increased significantly after the feeding only in group F. No differences were noted between groups in pre- and postprandial serum cortisol values. Similar findings were observed in the second experiment on d 6 (data not shown).

In the second experiment, there were no differences between groups in mean initial weight, final weight, weight gain, or formula intake (Table 2). Small intestinal length was similar in both groups (446 ± 23 versus 425 ± 17 cm/kg; F + I versus F, respectively). In contrast, small intestinal weight was greater in group F + I than in group F (41.1 ± 1.5 versus 37.0 ± 1.9 g/kg, $p < 0.001$). The difference in small intestinal weight resulted from increased ileal mucosal weight in group F + I compared with group F (Fig. 1). Serosal weight was similar between groups (data not shown). No differences were seen between groups in jejunal mucosal (Fig. 1) or serosal weights (data not shown).

No differences were seen between groups in total jejunal segment protein (Fig. 1). Similar findings were seen for total jejunal segment DNA concentration. Protein/DNA ratio was

similar between groups in the jejunum. Total jejunal segment RNA was not different between groups.

Because of the increase in ileal mucosal weight, total ileal mucosal segment protein was greater in group F + I than in group F (Fig. 1). Total ileal segment DNA was similar between groups. The ileal protein/DNA ratio was greater in group F + I than in group F. Total ileal segment RNA was greater in group F + I than in group F.

Although jejunal disaccharidase activity was similar between groups, ileal lactase and maltase activities (per kg body wt) were greater in group F + I than in group F (Fig. 2). When expressed per mg protein (Fig. 3), only ileal lactase activity was significantly greater in group F + I than in group F. No differences in ileal sucrase activity were noted between groups (Figs. 2 and 3).

DISCUSSION

Our study has demonstrated that ileal mass and lactase activity were increased in newborn miniature pigs in response to orally administered insulin. The increase was sufficiently large that total small intestinal mass was greater in group F + I than in group F. Previous *in vivo* studies have suggested that insulin administered parenterally to 8-d-old mice can stimulate the rate of enterocyte proliferation and cause a precocious increase in sucrase and maltase activities (10, 11, 17). Other studies in rats have contradicted aspects of the findings in mice (8, 9). *In vitro* studies of the effect of insulin on the small intestine have also produced conflicting results (3, 18–21). Contradictions among studies may be explained, in part, by differences in animal species, the ages of the animals studied, and the various dosages of insulin used. The interpretation of studies in which insulin was administered parenterally is difficult, because the animals often developed hypoglycemia (11). Hypoglycemia and its associated stress can stimulate ornithine decarboxylase activity, an enzyme intimately involved in small intestinal growth and development (22). We found no evidence in our study that orally administered insulin affected either serum glucose, insulin, or cortisol (Table 1). The 1-h blood sample should have detected significant changes in either serum glucose or insulin; the maximum increase in these parameters is found at 1 h during a glucose tolerance test (23).

In our attempt to interpret the effects of insulin on small intestinal growth and development in other animal studies, we found an additional confounding variable, *i.e.* animal food intake was not controlled (8, 9, 10, 11, 17). Insulin stimulates food intake, which in turn can increase small intestinal mass and disaccharidase activity (24, 25). By strictly controlling food intake in our study, we avoided this pitfall.

Studies in rats made diabetic suggest that the absence of insulin is associated with an increase in small intestinal mass and disaccharidase activity (26). The hyperphagia associated with diabetes in the rat, however, appears capable of inducing mucosal hypertrophy (24). In addition, diabetes appears to be a poor model in which to study the effects of insulin on the small intestine because the response may differ according to the etiology of the diabetic lesion (*e.g.* disaccharidase activity increased or unchanged) (26).

In a previous study, we administered different combinations of nutrients (glucose versus a combination of glucose and fat) *i.v.* to alter serum insulin levels in healthy newborn piglets (13). Although the piglets received no oral intake, they grew normally (13). We found no differences in small intestinal growth and development despite marked differences in serum insulin levels between groups. These data suggest that alterations in serum insulin in the absence of hypoglycemia and changes in food intake do not alter small intestinal growth and development. We thus chose to focus our attention on the possible effect of insulin administered orally with the feeding.

Studies of the effects of insulin administered orally may not have been undertaken previously because insulin administered in this manner is assumed to be degraded rapidly (27). Neverthe-

Table 1. Results of blood measurements*

Measurement	Formula + insulin	Formula
Serum glucose (mmol/L)		
Preprandial	4.2 $\pm$ 0.4	4.3 $\pm$ 0.5
Postprandial	10.6 $\pm$ 1.6	9.4 $\pm$ 0.7
Serum insulin (pmol/L)		
Preprandial	44 $\pm$ 7	72 $\pm$ 22
Postprandial	213 $\pm$ 90	163 $\pm$ 39
Serum cortisol (nmol/L)		
Preprandial	331 $\pm$ 119	270 $\pm$ 94
Postprandial	331 $\pm$ 124	389 $\pm$ 102

* Values are means $\pm$ SE. There were no differences between groups.

Table 2. Piglet wt, wt gain, and formula intake*

Parameter	Formula + insulin	Formula
Initial wt (g)	793.0 $\pm$ 53	754.0 $\pm$ 43
Final wt (g)	1024.0 $\pm$ 62	968.0 $\pm$ 52
Wt gain (g/kg/d)	44.6 $\pm$ 4.4	43.2 $\pm$ 3.7
Formula intake (mL/kg/d)	215.2 $\pm$ 6.5	222.1 $\pm$ 7.5

* Values are means $\pm$ SE.

Fig. 1. Hatched bars, formula and insulin; open bars, formula. Upper panel, jejunal segment measurements; Lower panel, ileal segment measurements. Values are means $\pm$ SE.

Fig. 2. Hatched bars, formula and insulin; open bars, formula. Upper panel, jejunal disaccharidase measurements expressed per kg body wt; Lower panel, ileal disaccharidase measurements expressed per kg body wt. Values are means $\pm$ SE.

less, pharmacologic doses of oral insulin (more than 170-fold higher than those used in our study) administered to piglets and human infants less than 24-h-old decreased serum glucose, presumably by having been absorbed (28, 29). This response does not occur in older animals (rats), even at pharmacologic doses (30). Such studies indicate that after the immediate newborn period, oral insulin does not induce systemic effects (*i.e.* decrease serum glucose). These data were substantiated in our present study, in which oral insulin did not affect serum glucose, insulin, or cortisol. The possibility remains, however, that small amounts of insulin might escape degradation and affect small intestinal mass and development. Further studies are warranted to test this hypothesis.

The hypothesis that oral insulin may affect small intestinal development and/or function is strengthened by the finding of insulin receptors on the jejunal and ileal brush border surfaces of rat small intestine (31, 32). In the dog, however, insulin

receptors appear to be much more numerous on the basolateral surface of jejunal enterocytes (33). In the rat, there appear to be no differences between jejunum and ileum in the specific binding of insulin to the receptors (32). Thus, we have yet to determine whether the insulin effects in our study occurred via these receptors.

If we assume that oral insulin did reach the small intestine intact, its effects might conceivably have been found in the jejunum. The effects of insulin added to the food, however, were observed in the ileum, but not the jejunum (Figs. 1–3). In the normal small intestine, jejunal villi are longer than ileal villi. This jejuno-ileal gradient appears to be intrinsically programmed (34). Thus, jejunal mass and enzyme activity may be maximally stimulated in the normal jejunum (34). An increase in the amount of nutrients and chyme within the lumen of the ileum, however, can increase ileal mass (35). Insulin may have altered motility in the jejunum so that a larger portion of the feed

Fig. 3. Hatched bars, formula and insulin; open bars, formula. Upper panel, jejunal disaccharidase measurements expressed per g protein; Lower panel, ileal disaccharidase measurements expressed per g protein. Values are means $\pm$ SE.

reached the ileum and induced mucosal hypertrophy. This would not explain the increase in ileal lactase activity (Figs. 2 and 3), which cannot be induced in the pig (and human) by changes in diet (36, 37). Alternatively, insulin may have elicited the release of factor(s), the effects of which are targeted to the ileum. In this regard, it is significant that the insulin treatment did not affect serum cortisol (Table 1), a hormone intimately involved in small intestinal development.

The mechanism by which insulin affects the ileum remains elusive. The increase in ileal mass cannot be explained by differences in tissue hydration, because the protein and RNA concentrations were not different between the two groups of piglets. Insulin may increase enterocyte life-span, perhaps by decreasing the rate of crypt cell production and the rate of cell loss. Tsuboi *et al.* (38) have suggested that the longer the life-span of the enterocyte, the greater the amount of lactase accreted by the cell. These data support the hypothesis that insulin prolongs enterocyte life-span, which accounts for the greater lactase activity (per g protein) seen in the F + I group compared with that in the F group (Fig. 3). Alternatively, insulin may alter the rate of protein synthesis and/or breakdown (39). The altered rate of protein turnover would be consistent with the effect insulin has on other tissues (39). The larger ileal protein/DNA ratio and higher total segment RNA in group F + I compared with that in group F (Fig. 1) support the latter speculation (40). The speculations, however, are not mutually exclusive; the similar concentrations and total amount per segment of DNA in the groups (Fig. 1) suggest that insulin did not increase cell number. Therefore, the change in ileal mass appears to be the result of hypertrophy rather than hyperplasia.

In summary, we have found that oral insulin administered to the newborn miniature piglet significantly increases small intestinal mass, ileal RNA, and lactase and maltase activities. These changes were unaccompanied by alterations in serum glucose, insulin, or cortisol. In future studies, it will be important to determine whether the amount of insulin that occurs naturally in colostrum is capable of producing the effects observed in our study. The amount of insulin we added to the formula (85 mU/

mL), although at least 150-fold less than that used in previous studies in which insulin was given parenterally, was greater than that found in pig colostrum (0.85 mU/mL) (6). Because of the important physiologic functions specific to the ileum (*e.g.* B₁₂ absorption), the dramatic increase in ileal mass and enzyme activities induced by insulin suggests a possible clinical application in patients whose intestinal function is limited, for example, premature infants.

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