0031-3998/01/4906-0782
PEDIATRIC RESEARCH
Vol. 49, No. 6, 2001
Printed in U.S.A.
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Development of Glucocorticoid-Responsiveness in Mouse Intestine

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ABSTRACT

There are conflicting data from human studies regarding the ability of exogenous glucocorticoids to stimulate maturation of the small intestine. The discrepancies may relate to differences in hormone doses and age administered. To explore this general concept, we have used a mouse model to determine intestinal responsiveness to dexamethasone (DEX) at various times during development. We first showed that trehalase mRNA is a sensitive marker of intestinal maturation in the mouse; being undetectable (by Northern blotting) in the prenatal period, expressed at low levels during the first 2 postnatal weeks and then displaying a marked increase in the 3rd postnatal week. DEX was unable to elicit detectable trehalase mRNA in fetal mice, but caused significant increases in the postnatal period. The use of a range of DEX doses (0.0125–2.5 nmol/g BW per day) established that there is no change in sensitivity between the 1st and 2nd postnatal weeks, but there is a significant increase in maximal responsiveness of trehalase mRNA to the hormone. Similar results were obtained when sucrase-isomaltase mRNA was assayed in the same animals. Thus, in this rodent model, there appears to be at least three phases in the DEX responsiveness of the developing intestine: an early phase (prenatal) when DEX is unable to elicit intestinal maturation; then a phase (first postnatal week) of modest responsiveness; then a transition to increased responsiveness. These findings point to the need for careful attention to dose and age in analyses of glucocorticoid effects in human infants. ***(Pediatr Res* 49: 782–788, 2001)**

Abbreviations

BW, body weight
DEX, dexamethasone
E, prenatal
P, postnatal
NEC, necrotizing enterocolitis

Glucocorticoids have become an important component in the pharmacologic treatment of prematurity because of their maturational effects on certain tissues. In the lung, for example, corticosteroid therapy is central to the prevention of respiratory distress syndrome. Interestingly, several clinical trials using exogenous glucocorticoids in the treatment of prematurity also demonstrated a decreased incidence of necrotizing enterocolitis (NEC) in steroid-treated infants (1–3). In addition, Halac and coworkers conducted a prospective human study of the effect of DEX on the incidence of NEC and found a significant reduction in NEC among neonates receiving either prenatal or postnatal steroid (4). As NEC is typically associated with immaturity (5–7), these findings suggest that exogenous glucocorticoid may exert a maturational effect on the intestine. However, not all trials observed decreased NEC (2). Likewise, although investigators have found significant maturation of intestinal motility (8) and permeability (9) in premature infants whose mothers received prenatal glucocorticoid, another study reported no effect on lactase maturation (10). The latter *in vivo* observation is in contrast to several studies that have shown elevation of lactase activity in response to glucocorticoid treatment in explant cultures of human fetal intestine (11–13). We hypothesize that such seemingly disparate results may be explained by differences in dosages and timing of corticosteroid treatment. In the current study we have used a mouse model to explore these issues.

Numerous studies over the past 4 decades have demonstrated maturational effects of glucocorticoids on the rodent intestine (14–17). Among these are several that point to age-dependent changes in responsiveness of the intestine to exogenous glucocorticoids (18–21). As with other hormones, such changes could reflect differences in sensitivity to low doses or in maximal responsiveness to saturating doses (22). Thus, a detailed understanding necessitates the use of a range of hormone doses at each age of interest. To date, most age-related studies of intestinal responsiveness to glucocorticoid have used a single dose of hormone (18, 19, 21). In addition, all of the

Received August 8, 2000; accepted January 9, 2001.
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Supported by grant HD 14094 from the National Institutes of Health.

prior rodent studies are confounded by the use of natural glucocorticoids whose circulating concentrations are profoundly influenced by corticosteroid-binding globulin. As the latter displays marked increases during the first 3 postnatal weeks (23, 24), a given dose of natural glucocorticoid at early ages yields very much lower circulating concentrations than does the same dose at later ages (25). This problem can be obviated by the use of a synthetic glucocorticoid such as dexamethasone (DEX), which does not bind to corticosteroid-binding globulin (26). Thus, a given dose of DEX yields the same circulating concentration at various developmental stages (25).

On the basis of the information above, the primary goal of the current study was to use a range of DEX doses to evaluate age-related changes in the glucocorticoid responsiveness of mouse intestine. We have focused our attention on the enzyme trehalase, a relatively less well-studied enzyme with growing importance in human nutrition (27–29). Because the developmental pattern of expression of trehalase mRNA has not been previously reported in mice, we first studied normal maturation to provide a physiologic context for precocious maturation elicited by DEX. Finally we compared the behavior of trehalase mRNA with that for sucrase-isomaltase mRNA to assess whether changes in DEX responsiveness are common or distinct.

MATERIALS AND METHODS

Animals and Tissue Collection

Timed-pregnant C57Bl/6J dams from Jackson Laboratory (Bar Harbor, ME) were received on the 12th or 13th day of gestation. They were housed individually in transparent polystyrene cages and provided with food (5001 Rodent Diet, PMI Nutrition International, Brentwood, MO, U.S.A.) and water *ad libitum*. Animals quarters were air-conditioned (21 ± 1°C) and maintained on a 12-h light/dark schedule with lights on at 0600 h. On the due date, cages were checked every 3 h for deliveries, and the birth date was regarded as day 0. On the 2nd postnatal day, the litters with more than seven pups were culled to seven pups. Pups remained with their dams for the remainder of the experiment. All animal protocols were approved by our institutional Animal Care and Use Committee. All injections were made s.c. once a day in the morning after weighing of the pups. Dexamethasone (DEX) was obtained from Sigma Chemical Co. (St. Louis, MO). Animals were *terminally* anesthetized with isoflurane for jejunum collection. The intestines of animals older than postnatal day 12 (P12) were flushed with ice-cold normal saline. All tissues were placed immediately in liquid nitrogen and were stored at −80°C.

RNA Isolation and Northern Blotting

Total cellular RNA from the jejunum was isolated using guanidine isothiocyanate extraction and pelleting through a cesium chloride cushion as previously described (30). Northern blots were generated according to a standard procedure (31) using 10 μg total RNA per lane. Each experiment included an adult mouse standard, which was a pooled RNA sample containing equal amounts of total RNA prepared from the jejunum of four C57Bl/6J male mice aged 6 wk. Probes used for Northern blotting were: rat trehalase cDNA (31), rat sucrase-isomaltase cDNA (32), mouse sucrase-isomaltase cDNA (Oesterreicher *et al.* unpublished), and mouse β-actin cDNA (33). The cDNA inserts were ^{32}P-labeled using the random primer method of Feinberg and Vogelstein (34). Prehybridization and hybridization were performed with a solution comprising 1 mM EDTA, 0.25M sodium phosphate pH 7.2, 7% SDS and 0.1 mg/mL denatured salmon sperm DNA. Blots were incubated with prehybridization solution at 65°C for at least 15 min before incubating with hybridization solution, which included the ^{32}P-labeled probe. After 16–18 h hybridization at 65°C, blots were washed twice at 65°C for 15 min in 1 mM EDTA, 20 mM sodium phosphate pH 7.2 and 5% SDS. If the background radioactivity signal was still high, an additional wash was performed at 65°C for 15 min in 1 mM EDTA, 20 mM sodium phosphate pH 7.2 and 1% SDS. Blots were then rinsed with 0.15 M NaCl/0.015 M sodium citrate. After initial probing with the cDNA of interest (trehalase or sucrase-isomaltase) blots were stripped (by heating at 90°C for 20 min with a solution containing 0.15 M NaCl/0.15 M sodium citrate and 0.5% SDS) and then reprobed with mouse β-actin as a constitutive marker.

Quantitative Analysis

Signals from Northern blots were quantified by phosphorimaging. To correct for loading variation, these data were expressed as a ratio of the hybridization signal of the band of interest (trehalase or sucrase-isomaltase) to that of the constitutive marker β-actin. This ratio was then expressed as a percent of adult mouse standard from the same blot. Statistical significance was assessed by one-way or two-way ANOVA followed by Fisher's LSD using Minitab program (Minitab Inc., PA, U.S.A.). For graphical presentation, values for individual animals from each experimental group were calculated as means ± SE. The number of animals per group is given in the figure legends.

Experimental Design

Study 1. Normal Development of Trehalase mRNA in Mouse Intestine. Small intestinal tissue was collected prenatally from three litters of mice and postnatally from four litters. The prenatal samples were at 15, 17, and 18 d of gestation and are designated E15, E17, and E18. At these ages the entire small intestine was used and tissue from four to eight fetuses was pooled to give sufficient RNA. Multiple litters were used to give at least two pools of tissue at each age. In the postnatal period, only the jejunum was collected and at each age a sample pup was taken from three or four different litters. RNA extraction and Northern blotting were as described above.

Study 2. Prenatal and Postnatal Responsiveness of Intestinal Trehalase mRNA to Exogenous Glucocorticoid. The primary rationale for using synthetic glucocorticoid for these studies has been given in the Introduction. Moreover, DEX is known to pass through the placenta of mice (35) and thus is suitable for prenatal administration *via* the mother. As the goal

of *Study 2* was to delineate appropriate ages for the subsequent dose response experiment, to minimize the number of mice used, this study used a single dose of DEX that was expected to yield maximal responsiveness on the basis of prior data in rats (36). The effect of DEX at the dose of 1 nmol (0.4 μg)/g body weight (BW) per day in the prenatal period was assessed by injecting two pregnant mice daily for 4 d from E14–17 and collecting the small intestine of fetuses at E18. Small intestine samples were pooled from 7–9 fetuses per dam. A control pregnant mouse was uninjected. The postnatal study was performed using DEX at the same dose injected to three mice from different litters starting at P8 for 3 d. Jejunums were collected at P11. RNA isolation and Northern blotting were performed as above.

Study 3. DEX Dose Response of Intestinal Trehalase mRNA in First and Second Postnatal Weeks. To establish the dose response to DEX, a littermate design was used. Each litter was treated as follows: one pup received normal saline as control, one pup received daily injection of DEX at 0.0125 nmol (0.005 μg)/g BW, one pup received DEX at 0.05 nmol (0.02 μg)/g BW, one pup received DEX at 0.25 nmol (0.1 μg)/g BW, one pup received DEX at 1nmol (0.4 μg)/g BW, one pup received DEX at 2.5 nmol (1.0 μg)/g BW and if there was an extra pup in the litter, this pup was uninjected. Injections were for 3 d beginning either at P2 (seven litters) or P8 (three litters). The jejunum was collected 1 day following the last injection (P5 and P11), respectively. The results of uninjected and normal saline injected groups were not significantly different, so data were pooled and are shown as zero dose.

Study 4. DEX Responsiveness of Intestinal Sucrase-Isomaltase mRNA. RNA samples from Studies 2 and 3 were re-run and the new Northern blots were probed with rat or mouse ^{32}P-labeled sucrase-isomaltase cDNA then stripped and re-probed with β actin.

RESULTS

Study 1. Normal Development of Trehalase mRNA in Mouse Intestine. The developmental expression of trehalase mRNA in mouse jejunum was assessed by collecting tissue at various prenatal and postnatal ages. As shown in Fig. 1*A*, Northern blotting revealed an abundant transcript in adult jejunum (*lane 1*). The size of this mouse mRNA was estimated to be approximately 2.3 kb in good agreement with that for trehalase mRNA in both rats (31) and rabbits (37). Trehalase mRNA was not detectable in prenatal animals (*lanes 2–4*) even after 5-fold longer exposure (not shown). In contrast, low levels were detected in the early postnatal period. The abundance of trehalase mRNA increased markedly during the 2nd and 3rd postnatal weeks and appeared to reach adult levels by P18 (Fig. 1*A*). Quantitative data from multiple animals at each age are shown in Fig. 1*B*. One-way ANOVA showed that the overall effect of age was highly significant ($p < 0.001$). *Post hoc* analysis revealed that values at P18 and P25 were not significantly different, indicating that the adult plateau is reached before the end of the 3rd postnatal week.

Study 2. Prenatal and Postnatal Responsiveness of Intestinal Trehalase mRNA to Exogenous Glucocorticoid. To delineate the range of ages to be included in the subsequent dose-response study, a single (high) dose of DEX was administered in the late fetal period or in the 2nd postnatal week. The results of Northern blot analysis are shown in Fig. 2. As can be seen, in the prenatal period, trehalase mRNA was undetectable in control animals (consistent with the previous study) and remained undetectable following DEX treatment. In contrast, in the 2nd postnatal week, low levels were seen for control animals and there was a marked increase (7-fold) in the levels of trehalase mRNA in response to DEX ($p < 0.001$). There was, in fact, full induction as the 11-day-old mice that had received glucocorticoid treatment displayed trehalase mRNA levels as high as those in adult animals.

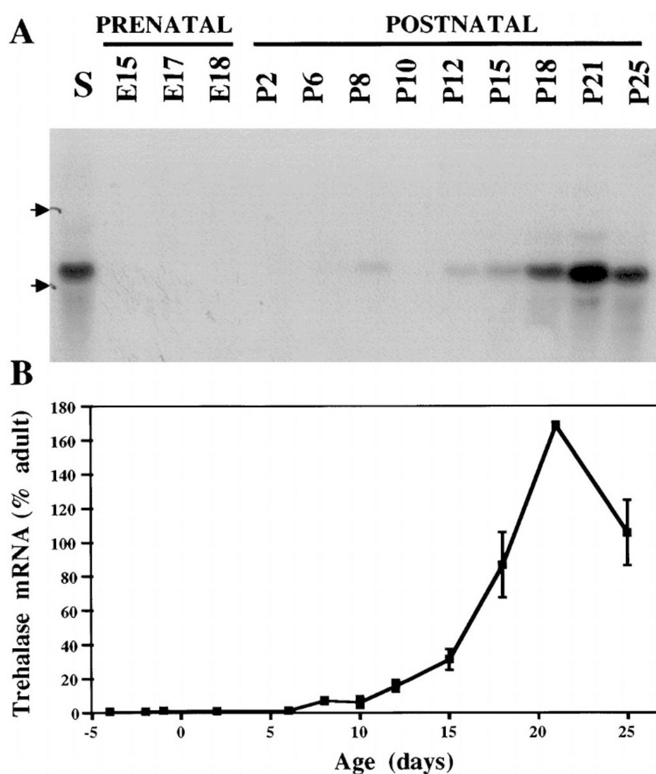

Figure 1. Normal development of trehalase mRNA in mouse intestine. *A*, Representative autoradiogram of Northern blot showing signal for trehalase mRNA, and positions of 18S and 28S rRNA (*arrows*). *Lane 1* (marked S) shows pooled adult standard, which was included on all blots. Remaining lanes show RNA collected at the prenatal and postnatal ages indicated. Prenatal ages are shown as days post-fertilization and postnatal ages are shown as days after birth. *B*, Quantitative data from all samples shown as means ± SEM for three to four pups at each age. Lack of error bars indicates that bars are smaller than the symbol.

Study 3. DEX Dose Response of Intestinal Trehalase mRNA in First and Second Postnatal Weeks. Based on results of Study 2, subsequent studies were focused on the postnatal period. To assess potential changes in both sensitivity and maximal responsiveness, a full range of DEX doses was administered during either the 1st or 2nd postnatal week. Daily weight gain of the pups was monitored to assess general systemic effects of the hormone treatment. Figure 3*A* shows that, as expected (38–40), there was a decrease in weight gain for pups receiving DEX in the 1st and 2nd postnatal weeks. At both ages, one-way ANOVA showed the overall effect of DEX

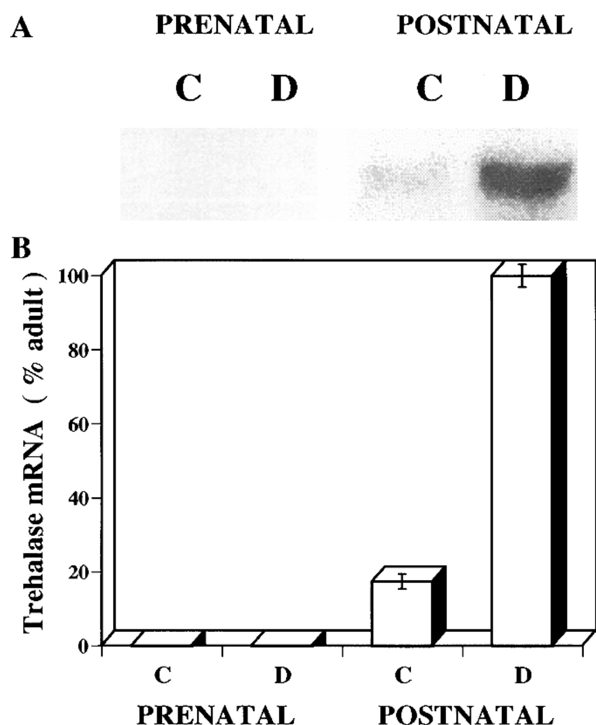

Figure 2. Comparison of glucocorticoid responsiveness of trehalase mRNA in prenatal and postnatal mice. *A*, Representative Northern blot of prenatal and postnatal trehalase mRNA comparing control (*C*) with DEX-treated animals (*D*). *B*, Quantitative data from all samples shown as mean ± SEM (n = 3).

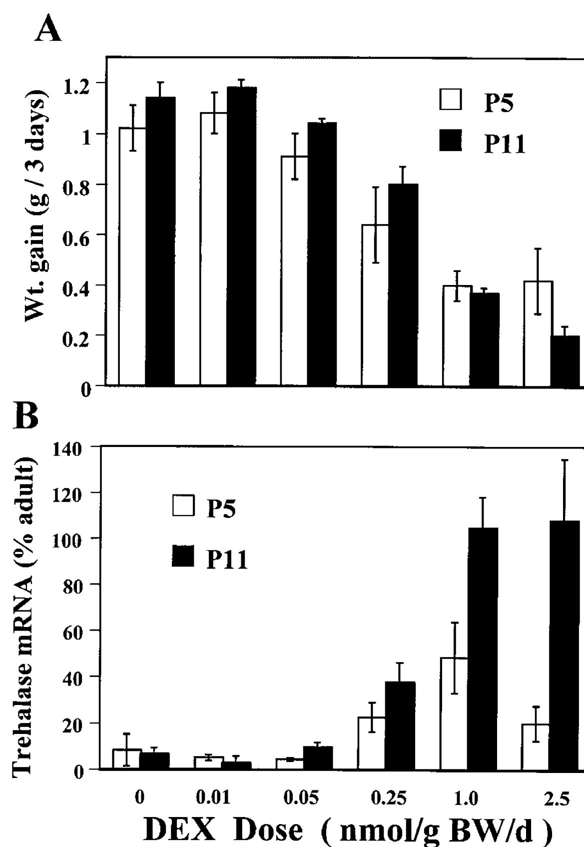

Figure 3. DEX dose-response of weight gain and trehalase mRNA in 1st and 2nd postnatal weeks. Animals received the DEX doses shown on lower axis either during the 1st postnatal week (P2–P4) or during the 2nd postnatal week (P8–P10). Data shown were collected at P5 and P11, respectively, and are given as mean ± SEM. The number of animals in each dosage group was seven for the P5 experiment and three for the P11 experiment. *A*, Weight gain from P2 to P5 (*open bars*) and from P8 to P11 (*solid bars*). *B*, Intestinal trehalase mRNA at P5 (*open bars*) and at P11 (*solid bars*).

dose to be significant ($p < 0.001$ in both cases) and *post hoc* analyses showed that the suppressive effect of DEX on weight gain becomes significant at the dose of 0.25 nmol (0.1 μg)/g BW and plateaus after 1 nmol (0.4 μg)/g BW.

The effects of increasing doses of DEX on the expression of trehalase mRNA during the 1st and 2nd postnatal weeks are shown in Fig. 3*B*. At both ages one-way ANOVA showed the overall effect of increasing doses of DEX to be highly significant ($p < 0.001$ in both cases). Just as with weight gain, *post hoc* analyses showed that the increase in trehalase mRNA becomes significant only at doses ≥ 0.25 nmol (0.1 μg)/g BW in both age groups. Thus there appears to be no difference in the sensitivity to DEX in the 2nd postnatal week as compared with the 1st postnatal week. In contrast, Fig. 3*B* shows that the maximal responsiveness increases markedly during this period (from 50% adult in the P5 pups to 100% in the P11 pups). When the effect of age was assessed by two-way ANOVA, the DEX responsiveness of trehalase mRNA was found to be significantly greater in the older animals ($p < 0.001$). There was also a significant interaction between DEX dose and age ($p < 0.001$) further substantiating the influence of age on DEX responsiveness.

Study 4. DEX Responsiveness of Intestinal Sucrase-Isomaltase mRNA. To assess whether the lack of DEX responsiveness in the prenatal period and the increased DEX responsiveness of trehalase mRNA in the 2nd postnatal week is gene specific or generic, the RNAs collected in Study 2 and Study 3 were analyzed for sucrase-isomaltase mRNA. Just as for trehalase mRNA, in the prenatal period, sucrase-isomaltase mRNA was undetectable by Northern blotting in both control mice and mice receiving DEX (data not shown). The results for the postnatal period are shown in Fig. 4. Overall the pattern was remarkably similar to that for trehalase mRNA with the response plateauing at the 1 nmol (0.4 μg)/g BW dose and with

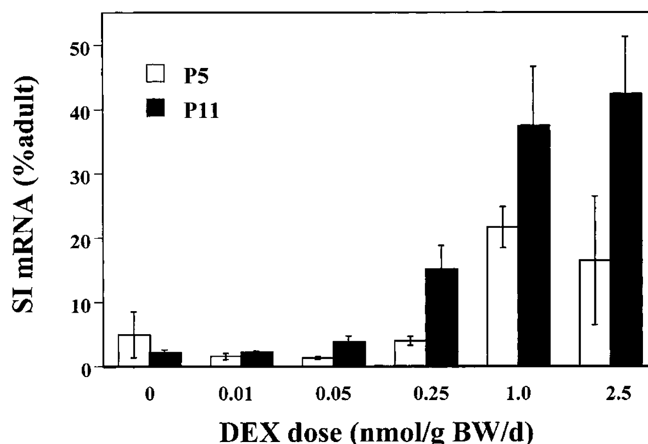

Figure 4. DEX dose-response of sucrase-isomaltase (SI) mRNA in 1st and 2nd postnatal weeks. Data are from the same animals as in Fig. 3 and are depicted in the same way.

the maximal response in the 2nd week being approximately double that in the 1st week. Most cogently, the two-way ANOVA showed a significant effect of both DEX dose and age ($p < 0.001$ in both cases) as well as a significant interaction between the two variables ($p < 0.002$). Thus, sucrase-isomaltase mRNA, like trehalase mRNA displays an age-related increase in the maximal responsiveness to DEX in the postnatal period.

Despite the similarities noted above, there were also subtle differences in the response of these two mRNAs to exogenous glucocorticoid. At both ages the overall responsiveness of sucrase-isomaltase mRNA was less than that of trehalase mRNA when compared with the respective adult reference ($p < 0.003$ at both ages). Thus, whereas DEX administration in the 1st postnatal week induced trehalase mRNA to 50% adult trehalase mRNA levels, the same animals had sucrase-isomaltase mRNA only at 22% adult sucrase-isomaltase mRNA levels. Likewise in the 2nd postnatal week DEX elicited higher levels of trehalase mRNA (100% adult) than of sucrase-isomaltase mRNA (41% adult). Overall these data indicate that although there is a generic age-related increase in the intestinal responsiveness to DEX, each mRNA has a characteristic degree of responsiveness as related to adult levels.

DISCUSSION

Glucocorticoids have been routinely used in clinical practice to prevent diseases of prematurity such as respiratory distress syndrome. There are reports that corticosteroid treatment may also reduce the incidence of NEC (1–3). As NEC is typically associated with immaturity of the intestine (5–7), the implication is that glucocorticoids enhance intestinal maturation. However, as delineated in the Introduction, direct assessment of glucocorticoid effects on specific aspects of intestinal maturation in humans have not yielded a clear picture. Although rodent intestine may differ from human intestine in the breadth of its responses to glucocorticoid (15), it is nevertheless a useful model to address issues related to dose and timing. To date numerous investigators have reported the ability of glucocorticoids to stimulate intestinal maturation in postnatal rodents (14–21), but questions regarding potential changes in either the sensitivity or the maximal responsiveness of the tissue have remained. The current study has addressed such questions using expression of trehalase mRNA and sucrase-isomaltase mRNA as indices of intestinal maturation. Trehalase was chosen because it has received the least attention of any of the disaccharidases, and yet may have growing importance in view of the proposed use of trehalose as an alternative sweetener to sucrose in a variety of manufactured foods (27, 28). Sucrase-isomaltase mRNA was compared with trehalase mRNA to determine whether the glucocorticoid effects are generic or gene specific.

As there were no data in the literature regarding the expression of trehalase mRNA in mice, our first goal was to study untreated animals to delineate the developmental pattern of trehalase mRNA in mouse intestine. Our results reveal that trehalase mRNA levels parallel the changes previously reported for trehalase enzyme activity (41) throughout development. As in rat (31) and rabbit (42), mouse trehalase mRNA levels rise significantly in the 3rd postnatal week during the weaning period. The rise of mouse trehalase mRNA seems to resemble that of sucrase-isomaltase mRNA (43). Although neither mRNA was detected in fetal intestine in the current study using Northern blots, other investigators, using the more sensitive method of RT-PCR have shown that both trehalase mRNA (Gartner and Henning, unpublished) and sucrase-isomaltase mRNA (43) are present at very low levels in the mouse intestine during the late fetal and early postnatal periods. Thus, the trehalase and sucrase-isomaltase genes may be similarly regulated during mouse development since both appear to be minimally transcribed in the fetal intestine then are markedly up-regulated during the 3rd postnatal week.

In contrast to the abundance of data demonstrating glucocorticoid responsiveness of rodent intestine in the postnatal period (14–17), there are conflicting reports relating to the fetal period. Despite the presence of the glucocorticoid receptor in fetal rat intestine (44), Lebenthal found no effect of a pharmacologic dose of hydrocortisone (50 μg/g BW) on sucrase activity (45). Using even higher doses of cortisone acetate, Jumawan *et al.* (46) observed very modest increases in sucrase activity (to 3% adult levels). Our study used a DEX dose range that is in the same range as that used in several clinical trials (1–3). We did not detect an effect of DEX on fetal mouse intestinal trehalase mRNA nor on sucrase-isomaltase mRNA despite the fact that, as noted above, both of these genes are already being transcribed at this time. Interestingly fetal mouse intestine in organ culture shows small increases of trehalase, maltase, and sucrase activities in response to DEX at a concentration calculated to be similar to circulating concentrations in our study (47). Because the increase disaccharidase activity was seen only *in vitro*, our inability to measure an *in vivo* response may support the existence of a circulating or intraluminal factor that can inhibit induction. Alternatively, there may be a very small *in vivo* response of fetal mouse intestine that is below the limit of detection of our Northern blot analysis and is unlikely to be of functional significance.

Previous studies have shown that trehalase enzyme activity in mouse small intestine is markedly increased by cortisone treatment during the 2nd postnatal week (48). The current work extends this observation to the mRNA level and shows that significant glucocorticoid responsiveness can also be observed during the 1st postnatal week. At both ages (Fig. 3), the use of DEX at a range of doses allowed assessment of both sensitivity and maximal responsiveness. Comparing the effect of DEX in the 1st and 2nd postnatal weeks showed no influence of age on sensitivity to this synthetic glucocorticoid. At both ages the lowest dose of DEX capable of eliciting a significant increase in trehalase mRNA or sucrase-isomaltase mRNA was 0.25 nmol (0.1 μg)/g BW/d. These results are quite different from those in rat using cortisone acetate, which led to the conclusion that glucocorticoid sensitivity increases between the 1st and 2nd postnatal weeks (20). As noted earlier we believe these rat data are confounded by the use of a natural steroid that binds to corticosteroid-binding globulin. Our findings with DEX are consistent with mediation by the glucocorticoid receptor whose

constant affinity for a given corticosteroid (44) would result in tissue sensitivity being unaffected by developmental age.

Contrasting with the sensitivity, our results clearly show that, for both trehalase mRNA and sucrase-isomaltase mRNA, maximal responsiveness to DEX increased from the 1st to the 2nd postnatal week. Although developmental changes in glucocorticoid receptor density would provide a possible explanation for the increased maximal responsiveness, a study in rat demonstrated that the receptor abundance does not increase from the 1st to the 2nd postnatal week (44). The observed increases in steady state levels of trehalase mRNA and sucrase-isomaltase mRNA could reflect an influence of age on either gene transcription or mRNA stability. Experiments to distinguish between these possibilities were beyond the scope of the current study. Other investigators have shown that for both mouse trehalase mRNA (49) and rat sucrase-isomaltase mRNA (50), the levels induced by glucocorticoids in the 2nd postnatal week can be entirely accounted for by increased transcription. Regardless of the molecular basis, a plausible cause of the enhanced DEX responses in older animals may be the increase of circulating thyroxine that occurs in the 2nd postnatal week (51). Synergistic effects of DEX and thyroxine on the levels of rat sucrase-isomaltase mRNA during the 2nd postnatal week have been shown previously (52, 53).

Although not one of the specific goals of this study, our data (Fig. 3) showing decreased body weight gain in proportion to DEX dose confirm prior reports of glucocorticoid inhibition of growth in postnatal rats (38, 39). Adverse effects of glucocorticoids on body weight have also been previously reported in prenatal rodents (40, 54) and in postnatal human infants (55–58). The explanation of the negative effect on growth is probably multifactoral. Animal studies have shown that glucocorticoid treatment results in decreased DNA and protein synthesis (35, 39, 40, 54, 56), which may in turn be due to decreased action of anabolic hormones such as GH, insulin, and IGF-1 (39, 40, 54, 59). In addition, studies in preterm babies have shown that DEX treatment has a distinct catabolic effect on muscle tissue (60). For the purposes of our study, the most interesting aspect of the weight gain data in Fig. 3 is that the magnitude of the DEX effects were not influenced by age. This suggests that the increased responsiveness observed in the 2nd postnatal week for both trehalase mRNA and sucrase-isomaltase mRNA reflects specific enhancement of glucocorticoid action in the small intestine as compared with other organs and tissues.

The observed pattern of intestinal responsiveness to DEX administration during prenatal and postnatal development is consistent with responses to endogenous glucocorticoid at the equivalent stages. There are two surges of circulating corticosterone during rodent development: one occurs toward the end of gestation (61) and the second begins at the end of the 2nd postnatal week (23, 62). Interestingly, peak levels of corticosterone reported prenatally (61) are at least 4-fold higher than those observed in the 3rd postnatal week (23, 62). Nevertheless the intestine shows no prenatal response as judged by the fact that both trehalase mRNA (Fig. 1) and sucrase-isomaltase mRNA (43) in the fetal mouse intestine remain below the limit of detection by Northern blotting. In contrast, in the postnatal mouse our observed increase in DEX responsiveness in the 2nd postnatal week correlates with the beginning of the normal developmental increase of expression of both trehalase mRNA (Fig. 1) and sucrase-isomaltase mRNA (43).

In addition to the findings reported here regarding lack of responsiveness to glucocorticoid in the prenatal period and increasing responsiveness during the 1st and 2nd postnatal weeks, there is also extensive evidence (15, 16) that the rodent intestine becomes unresponsive to glucocorticoid from the 3rd postnatal week onward. Taken together, these data indicate that during rodent development there is a relatively narrow "window" during which intestinal maturation can be enhanced by exogenous glucocorticoid. The possible existence of a similar window of responsiveness during human development (8) has important implications both for the interpretation of existing human studies and the design of future trials. Furthermore, just as has been postulated for the developing brain (63), it is likely that different aspects of intestinal function may display distinct critical windows. Such a scenario would explain the observation that antenatal corticosteroid was associated with maturation of intestinal permeability (9) but had not effect on lactase activities (10) in the same group of premature infants. Purposeful inclusion of a range of gestational ages and multiple markers of intestinal maturation would be desirable features of future human trials.

Acknowledgments. The authors thank The Texas Children's Hospital Cancer Center and the Children's Nutrition Research Center for the use of phosphorimagers; Dr. Nanda Nanthakumar who performed preliminary studies in rats, which highlighted the need for the current study; Sylvia James and Juan Diaz for assistance with studies 1 and 2; Drs. Sandy Hwang and Joel Solomon for encouragement and advice; and Dr. Robert Shulman for his helpful comments on the manuscript.

REFERENCES

1. Bauer CR, Morrison JC, Poole WK, Korones SB, Boehm JJ, Rigatto H, Zachman RD 1984 A decreased incidence of necrotizing enterocolitis after prenatal glucocorticoid therapy. Pediatrics 73:682–688
2. Crowley P, Chalmers I, Keirse MJNC 1990 The effects of corticosteroid administration before preterm delivery: an overview of the evidence from controlled trials. Br J Obstet Gynecol 97:11–25
3. Tapia JL, Ramírez R, Cifuentes J, Fabres J, Hübner ME, Bancalari A, Mercado ME, Standen J, Escobar M 1998 The effect of early dexamethasone administration on bronchopulmonary dysplasia in preterm infants with respiratory distress syndrome. J Pediatr 132:48–52
4. Halac E, Halac J, Bégué EF, Casanas JM, Indiveri DR, Petit JF, Figueroa MJ, Olmas JM, Rodriguez LA, Obregon RJ, Martinez MV, Grinblat DA, Vilarrodona HO 1990 Prenatal and postnatal corticosteroid therapy to prevent neonatal necrotizing enterocolitis: a controlled trial. J Pediatr 117:132–138
5. Crissinger KD 1995 Animal models of necrotizing enterocolitis. J Pediatr Gastroenterol Nutr 20:17–22
6. Kliegman R 1990 Models of the pathogenesis of necrotizing enterocolitis. J Pediatr 117:S2–S5
7. Israel EJ 1994 Neonatal necrotizing enterocolitis, a disease of the immature intestinal mucosal barrier. Acta Paediatr Suppl 396:27–32
8. Morriss FH, Moore M, Weisbrodt NW, West MS 1986 Ontogenic development of gastrointestinal motility: IV. Duodenal contractions in preterm infants. Pediatrics 78:1106–1113
9. Shulman RJ, Schanler RJ, Lau C, Heitkemper MA, Ou C, Smith EO 1998 Early feeding, antenatal glucocorticoids, and human milk decrease intestinal permeability in preterm infants. Pediatr Res 44:519–523
10. Shulman RJ, Schanler RJ, Lau C, Heitkemper M, Ou C, Smith EO 1998 Early feeding, feeding tolerance, and lactase activity in preterm infants. J Pediatr 133:645–649
11. Villa M, Ménard D, Semenza G, Mantei N 1992 The expression of lactase enzymatic activity and mRNA in human fetal jejunum. FEBS Lett 301:202–206

12. Arsenault P, Ménard D 1985 Influence of hydrocortisone on human fetal small intestine in organ culture. J Pediatr Gastroenterol Nutr 4:893–901
13. Simon-Assmann P, Lacroix B, Kedinger M, Haffen K 1986 Maturation of brush border hydrolases in human fetal intestine maintained in organ culture. Early Hum Dev 13:65–74
14. Henning SJ 1981 Postnatal development: coordination of feeding, digestion, and metabolism. Am J Physiol 241:G199-G214
15. Henning SJ, Rubin DC, Shulman RJ 1994 Ontogeny of the intestinal mucosa. In: Johnson LR (ed) Physiology of the Gastrointestinal Tract, 3rd Ed, Raven Press, New York, pp 571–610
16. Galand G 1989 Brush border membrane sucrase-isomaltase, maltase-glucoamylase and trehalase in mammals. Comp Biochem Physiol 94B:1–11
17. Moog F 1971 Corticoids and the enzymic maturation of the intestinal surface: alkaline phosphataze, leucyl naphthylamidase, and sucrase. In: Hamburgh M, Barrington EJW (eds) Hormones in Development. Appleton-Century-Crofts, East Norwalk, CT, pp 143–160
18. Moog F, Denes AE, Powell PM 1973 Disaccharidases in the small intestine of the mouse: normal development and influence of cortisone, actinomycin D, and cycloheximide. Dev Biol 35:143–159
19. Henning SJ, Helman TA, Kretchmer N 1975 Studies on normal and precocious appearance of jejunal sucrase in suckling rats. Biol Neonate 26:249–262
20. Herbst JJ, Koldovsky O 1972 Cell migration and cortisone induction of sucrase activity in jejunum and ileum. Biochem J 126:471–476
21. Yeh K-Y, Yeh M, Holt PR 1991 Thyroxine and cortisone cooperate to modulate postnatal intestinal enzyme differentiation in the rat. Am J Physiol 260:G371–G378
22. Bamberger CM, Schulte HM, Chrousos GP 1996 Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. Endocr Rev 17:245–261
23. Henning SJ 1978 Plasma concentrations of total and free corticosterone during development in the rat. Am J Physiol 235:E451–E456
24. Scrocchi LA, Hearn SA, Han VKM, Hammond GL 1993 Corticosteroid-binding globulin biosynthesis in the mouse liver and kidney during postnatal development. Endocrinology 132:910–916
25. Schroeder RJ, Henning SJ 1989 Roles of plasma clearance and corticosteroid-binding globulin in the developmental increase in circulating corticosterone in infant rats. Endocrinology 124:2612–2618
26. Mickelson KE, Westphal U 1980 Steroid-protein interactions. Influence of steroid structure and temperature on the binding of steroids to guinea pig corticosteroid-binding globulin. Biochemistry 19:585–590
27. Newman YM, Ring SG, Colaco C 1993 The role of trehalose and other carbohydrates in biopreservation. Biotechnol Genet Eng Rev 11:263–294
28. Paiva CL, Panek AD 1996 Biotechnological applications of the disaccharide trehalose. Biotechnol Annu Rev 2:293–314
29. Sato S, Okamoto K, Minami R, Kohri H, Yamamoto S 1999 Trehalose can be used as a parenteral saccharide source in rabbits. J Nutr 129:158–164
30. Leeper LL, Henning SJ 1990 Development and tissue distribution of sucrase-isomaltase mRNA in rats. Am J Physiol 258:G52–G58
31. Oesterreicher TJ, Nanthakumar NN, Winston JH, Henning SJ 1998 Rat trehalase: cDNA cloning and mRNA expression in adult rat tissues and during intestinal ontogeny. Am J Physiol 274:R1220–R1227
32. Chandrasena G, Sunitha I, Lau C, Nanthakumar NN, Henning SJ 1992 Expression of sucrase-isomaltase mRNA along the villus-crypt axis in the rat small intestine. Cell Mol Biol 38:243–254
33. Alonso S, Minty A, Bourlet Y, Buckingham M 1986 Comparison of three actin-coding sequences in the mouse; evolutionary relationships between the actin genes of warm-blooded vertebrates. J Mol Evol 23:11–22
34. Feinberg AP, Vogelstein B 1983 A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13
35. Tye LM, Burton AF 1980 Effects of dexamethasone on fetal mouse development. Biol Neonate 38:260–264
36. Nanthakumar NN, Henning SJ 1993 Ontogeny of sucrase-isomaltase gene expression in rat intestine: responsiveness to glucocorticoids. Am J Physiol 264:G306–G311
37. Ruf J, Wacker H, James P, Maffia M, Seiler P, Galand G, von Kieckebusch A, Semenza G, Mantei N 1990 Rabbit small intestinal trehalase. J Biol Chem 265:15034–15039
38. Gramsbergen A, Mulder EJH 1998 The influence of betamethasone and dexamethasone on motor development in young rats. Pediatr Res 44:105–110
39. Beckett PR, Fiorotto ML, Davis TA, Reeds PJ 1996 Corticosterone has independent effects on tissue maturation and growth in the suckling rat. Pediatr Res 39:395–400
40. Frank L, Roberts RJ 1979 Effects of low-dose prenatal corticosteroid administration on the premature rat. Biol Neonate 36:1–9
41. Menard D, Malo C 1978 Glucose-6-phosphatase activity in mouse small intestine during postnatal development. Dev Biol 65:508–514
42. Galand G, L'Horset F, Longis Y, Perret C 1995 Trehalase gene expression during postnatal development of rabbit intestine and kidney: effects of glucocorticoids. Am J Physiol 269:G833–G841
43. Tung J, Markowitz AJ, Silberg DG, Traber PG 1997 Developmental expression of SI is regulated in transgenic mice by an evolutionarily conserved promoter. Am J Physiol 273:G83–G92
44. Henning SJ, Ballard PL, Kretchmer N 1975 A study of the cytoplasmic receptors for glucocorticoids in intestine of pre- and postweanling rats. J Biol Chem 250:2073–2079
45. Lebenthal E 1977 Induction of fetal rat enterokinase (enteropeptidase EC.3.4.21.9) *in utero* by hydrocortisone and actinomycin D. Pediatr Res 11:282–285
46. Jumawan J, Celano P, Horowitz C, Lau H, Koldovsky O 1977 Effect of cortisone or *L*-triiodothyronine administration to pregnant rats on the activity of fetal intestinal disaccharidases and lysosomal acid β-galactosidase. Biol Neonate 32:211–217
47. Beaulieu J-F, Calvert R 1985 Influences of dexamethasone on the maturation of fetal mouse intestinal mucosa in organ culture. Comp Biochem Physiol 82A:91–95
48. Malo C, Ménard D 1979 Effects of cortisone and thyroxine on intestinal trehalase activity in infant mouse. Experientia 35:874–875
49. Oesterreicher TJ, Markesich DC, Shukla P, Gartner H, Henning SJ 2000 Structure and hormonal regulation of the mouse trehalase gene. Gastroenterology 118:A293
50. Yeh K-Y, Yeh M, Glass J 2000 Glucocorticoids and dietary iron regulate postnatal intestinal heavy and light ferritin expression in rats. Am J Physiol Gastrointest Liver Physiol 278:G217–G226
51. Walker P, Dubois JD, Dussault JH 1980 Free thyroid hormone concentrations during postnatal development in the rat. Pediatr Res 14:247–249
52. Yeh K-Y, Yeh M, Montgomery RK, Grand RJ, Holt PR 1991 Cortisone and thyroxine modulate intestinal lactase and sucrase mRNA levels and activities in the suckling rat. Biochem Biophys Res Commun 180:174–180
53. Leeper LL, McDonald MC, Heath JP, Henning SJ 1998 Sucrase-isomaltase ontogeny: Synergism between glucocorticoids and thyroxine reflects increased mRNA and no change in cell migration. Biochem Biophys Res Commun 246:765–770
54. Celsi G, Kistner A, Aizman R, Eklöf A-C, Caccatelli S, de Santiago A, Jacobson SH 1998 Prenatal dexamethasone causes oligonephronia, sodium retention, and higher blood pressure in the offspring. Pediatr Res 44:317–322
55. Yeh TF, Torre JA, Rastogi A 1990 Early postnatal dexamethasone therapy in premature infants with severe respiratory distress syndrome: A double-blind, controlled study. J Pediatr 117:273–282
56. Taeusch HW 1975 Glucocorticoid prophylaxis for respiratory distress syndrome: a review of potential toxicity. J Pediatr 87:617–623
57. Harkavy KL, Scanlon JW, Chowdhry PK, Grylack LJ 1989 Dexamethasone therapy for chronic lung disease in ventilator- and oxygen-dependent infants: a controlled trial. J Pediatr 115:979–983
58. Peng CT, Lin HC, Lin YJ, Tsai CH, Yeh TF 1999 Early dexamethasone therapy and blood cell count in preterm infants. Pediatrics 104:476–481
59. Ward WE, Donovan SM, Atkinson SA 1998 Dexamethasone-induced abnormalities in growth and bone metabolism in piglets are partially attenuated by growth hormone with no synergistic effect of insulin-like growth factor-I. Pediatr Res 44:215–221
60. Brownlee KG, Ng PC, Henderson MJ, Smith M, Green JH, Dear PRF 1992 Catabolic effect of dexamethasone in the preterm baby. Arch Dis Child 67:1–4
61. Martin CE, Cake MH, Hartmann PE, Cook IF 1977 Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. Acta Endocrinologia 84:167–176
62. Diez JA, Sze PY, Ginsburg BE 1976 Postnatal development of mouse plasma and brain corticosterone levels: new findings contingent upon the use of a competitive protein-binding assay. Endocrinology 98:1434–1442
63. Matthews SG 2000 Antenatal glucocorticoids and programming of the developing CNS. Pediatr Res 47:291–300