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Dexamethasone and GLP-2 administered to rat dams during pregnancy and lactation have late effects on intestinal sugar transport in their postweanling offspring

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Abstract

Intestinal function in young animals is influenced by maternal factors, such as alterations in the maternal diet. Glucagon-like peptide 2 (GLP-2) enhances intestinal growth and absorption in mature animals. Glucocorticosteroids induce intestinal maturation in neonates and increase sugar uptake in adult animals. It is not known if maternally administered GLP-2 or glucocorticosteroids have persistent effects on intestinal transport in the offspring. This study was undertaken to determine (1) the influence of maternal GLP-2, dexamethasone (DEX) and GLP-2+DEX on intestinal sugar uptake in postweanling offspring and (2) if alterations in uptake are due to variations in intestinal morphology, sugar transporter abundance or the abundance of selected signals. Nursing rat dams were treated during pregnancy and lactation with GLP-2 (0.1 μg/g per day sc), DEX (0.128 μg/g per day sc), GLP-2+DEX or placebo. The offspring were sacrificed 4 weeks after weaning, and glucose and fructose uptake was determined using an in vitro intestinal ring uptake technique. sodium-dependent glucose transporter, glucose transporter (GLUT) 5, GLUT2, sodium potassium adenosine triphosphatase and selected signals were assessed by immunohistochemistry. The treatments did not affect body weights or intestinal morphology. GLP-2 and GLP-2+DEX increased jejunal fructose uptake, and GLP-2+DEX increased the jejunal and ileal maximal transport rate for glucose uptake. Protein kinase B and mammalian target of rapamycin abundance were also increased, while transporter abundance was unchanged. We speculate that these alterations in sugar uptake may be due to changes in the intrinsic activity of the transporters mediated by the phosphatidylinositol-3-kinase pathway. These alterations in uptake may have nutritional implications for the offspring of mothers who may be treated with GLP-2 or glucocorticosteroids.

Keywords: Adaptation; Programming; Glucose; GLUT2; GLUT5; SGLT1

1. Introduction

"Critical period programming" is a phenomenon by which a biological mechanism is irreversibly turned on or off once during a lifetime in response to prevailing conditions at a critical stage [1]. This concept, which has also been referred to as "metabolic programming" or "imprinting" [2,3], has been used to explain associations between prenatal/neonatal environmental events, alterations in growth and development and later pathophysiology [4,5].

The ontogeny of the intestine may be influenced by early exposure to nutrients. Several studies have documented changes in intestinal function as a result of alterations in the lipid or carbohydrate content of the weanling diet [1,6–12]. For example, Thomson and Keelan [11] demonstrated

Abbreviations: Akt/PKB, Protein kinase B; BBM, brush border membrane; BLM, basolateral membrane; DEX, dexamethasone; EGF, epidermal growth factor; GC, glucocorticosteroids; GLP-2, glucagon-like peptide-2; GLUT, glucose transporter; GSK-3, glycogen synthase kinase 3; IGF, insulinlike growth factor; Km, apparent affinity constant; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; Na^+K^+-ATPase, sodium potassium adenosine triphosphatase; NOS3, nitric oxide synthase 3; PCNA, proliferating cell nuclear antigen; PI3K, phosphatidylinositol-3-kinase; PKA, protein kinase A; PKC, protein kinase C; SGLT1, sodium-dependent glucose transporter; V_{max}, maximal transport rate.

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that feeding 8-week-old rabbits a low-cholesterol diet for 2 weeks reduced intestinal glucose uptake and that this effect persisted for at least 10 weeks.

Maternal diets also influence intestinal function. For example, feeding diets enriched in saturated fatty acids to lactating rat dams resulted in increases in sugar uptake in the weanling offspring [8]. Curiously, these changes were not seen in the suckling offspring, suggesting that the mechanisms responsible for adaptation may not be fully developed in these animals. Jarocka-Cyrta et al. [13] showed that feeding diets differing in fatty acid composition to pregnant and lactating rat dams influenced intestinal function in their offspring. These studies demonstrate that the programming of intestinal function begins very early in life, perhaps even in utero.

While early diet clearly influences intestinal transport later in life, it is also possible that other factors may influence intestinal function. Several hormones and growth factors have been shown to cause precocious intestinal development and to potentially stimulate intestinal growth and nutrient transport. However, it is not known if early exposure to these factors has lasting effects on intestinal function.

The enhancing effect of glucagon-like peptide-2 (GLP-2) on intestinal sugar absorption in adult animals is well documented [14–16]. However, it is not known if GLP-2 influences the intestinal absorption of sugars in young animals. Similarly, glucocorticosteroids (GC) are known to increase the uptake of both sugars and lipids in mature animals [17,18], but the effects of maternal GC on intestinal absorptive function in the offspring have not been studied. Furthermore, GC increase apoptosis [19], whereas GLP-2 increases proliferation and decreases apoptosis in the intestine of adult animals [20–22]. These alterations may indirectly influence sugar absorption by altering the population of mature transporting enterocytes. GC may be administered clinically to pregnant and lactating mothers, and it is not known if this affects sugar absorption in their offspring, or if any possible effects persist after GC are discontinued. Also, it is not known if the effects of GC can be modified by the coadministration of GLP-2 or if GLP-2 itself given during pregnancy and lactation alters intestinal sugar absorption in the offspring.

The regulation of intestinal sugar uptake is complex and may involve a number of intracellular signals and signaling pathways. In vitro studies demonstrate that protein kinase C (PKC) and protein kinase A (PKA) increase or decrease intestinal glucose uptake, respectively [23,24]. In vivo studies using inhibitors of phosphatidylinositol-3-kinase (PI3K) show that this pathway is important in both insulinlike growth factor (IGF)-1 and epidermal growth factor (EGF) stimulated intestinal glucose uptake [25,26]. The PKCβ-mediated recruitment of glucose transporter (GLUT) 2 to the brush border membrane (BBM) involves the mitogen-activated protein kinase (MAPK) pathway and the PI3K pathway [27]. Furthermore, although the effect of dexamethasone (DEX) on the PI3K pathway is unknown, GLP-2 induced intestinal proliferation is PI3K dependent [28,29]. Finally, GLP-2 has been shown to influence glycogen synthase kinase 3 (GSK-3) and endothelial nitric oxide synthase [nitric oxide synthase 3 (NOS3)] in total parenteral nutrition (TPN)-fed piglets, which may indirectly impact upon intestinal sugar uptake via effects on intestinal blood flow and cell proliferation [21,30].

Accordingly, this study was undertaken to determine (1) the influence of GLP-2, dexamethasone (DEX) and GLP-2+DEX when administered to pregnant and lactating rat dams, on the intestinal in vitro uptake of glucose and fructose in their postweanling offspring and (2) if alterations in the uptake of sugars are due to variations in intestinal morphology, the abundance of the sugar transporters or the abundance of selected signals known to regulate sugar transport.

2. Materials and methods

2.1. Animals

The Principles for the Care and Use of Laboratory Animals, approved by the Canadian Council on Animal Care and by the Council of the American Physiological Society, were observed in the conduct of this study. All experiments were approved by the Animal Ethics Board, University of Alberta. Eight 1-week-old pregnant Sprague–Dawley rats were obtained from Bio Science Animal Services, University of Alberta. The dams were randomized into four groups which received treatment with GLP-2, DEX, GLP-2 plus DEX or placebo. Treatment was started 10 days before delivery and was continued until the offspring were weaned at 19–21 days of age (Fig. 1). DEX was administered in a dose of 0.128 μg/g body weight per day sc once per day at 7 PM. GLP-2 was administered in a dose of 0.1 μg/g body weight per day sc twice per day at 7 AM and 7 PM. The regimen used for DEX+GLP-2 group was DEX 0.128 μg/g body weight per day sc once per day at 7 PM plus GLP-2 0.1 μg/g body weight per day sc twice per day at 7 AM and 7 PM. The doses chosen were based on studies done by Scott et al. [31] and Park et al. [32]. The placebo group received 0.9% saline sc in a volume equal to the volume used for GLP-2 administered daily per rat, twice per day at 7 AM and 7 PM.

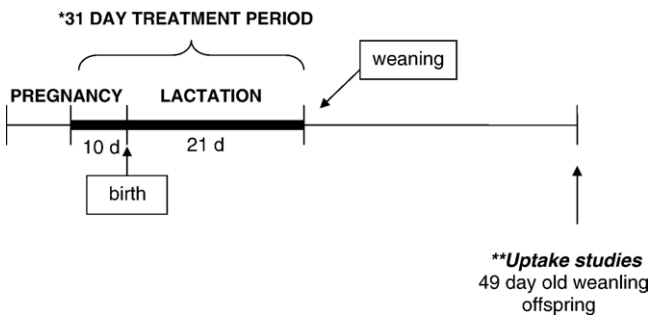

Fig. 1. Experimental design.

After delivery, the number of offspring was down-sized to 12 pups, which were housed with their dams. This resulted in two dams and 24 pups in each group. Postweaning animals ("postweanlings") were sacrificed for uptake studies at 7 weeks of age.

The animals were housed at a temperature of 21°C, and each day, they were exposed to 12 h of light and 12 h of darkness. During the suckling period, the offspring received only the dam's milk. The postweanling animals were housed in pairs. Their water and food were supplied ad libitum. The dams and the postweanlings were fed standard rat chow PMI#5001 (Nutrition International, Brentwood, MO, USA). The diets were nutritionally adequate, providing for all known essential nutrient requirements. Body weights were recorded at the time of weaning and then weekly for the next 4 weeks.

2.2. Uptake studies

2.2.1. Probe and marker compounds

The [^{14}C]-labelled probes included a range of concentrations of glucose (2–64 mM) and fructose (4–64 mM). The labeled and unlabeled probes were supplied by Amersham Biosciences (Baie d'Urfe, PQ, Canada) and Sigma (St. Louis, MO, USA), respectively. [^{3}H]-inulin was used as a nonabsorbable marker to correct for the adherent mucosal fluid volume [33].

2.2.2. Tissue preparation

Eight animals per treatment group were sacrificed by an intraperitoneal injection of Euthanyl (sodium pentobarbital, 240 mg/100 g body weight). The whole length of the small intestine was rapidly removed and rinsed with 150 ml cold saline. The intestine was divided into two parts: the proximal half of the intestine beginning at the ligament of Treitz was termed the *jejunum*, and the distal half was termed the *ileum*. A 2-cm piece of each segment of jejunum and ileum was gently scraped with a glass slide. The mucosal scrapings and the remaining wall of the intestine were dried overnight in an oven at 55°C. The percentage of the intestinal wall comprised of mucosa was calculated. The remaining intestine was everted and cut into small rings of approximately 2–4 mm each. These intestinal rings were immersed in preincubation beakers containing Krebs's buffer (pH 7.2) at 37°C, bubbled with oxygen plus carbon dioxide (O_2-CO_2, 95:5 by volume) and were allowed to equilibrate for 5 min [33]. Uptake was initiated by the timed transfer of the tissue rings from the preincubation buffer to a 5-ml plastic vial containing [^{3}H]-inulin and ^{14}C-labelled sugars in Krebs buffer bubbled with oxygen plus bicarbonate that had been equilibrated to 37°C in a shaking water bath. The intestinal rings were incubated in the substrates for 5 min.

2.2.3. Determination of uptake rates

The rate of sugar uptake was terminated by pouring the vial contents onto filters on an Amicon vacuum filtration manifold that was maintained under suction, followed by washing the intestinal rings three times with ice-cold saline. The tissue rings were placed on a glass slide and were dried overnight in an oven at a constant temperature of 55°C. The dry weight of the tissue was determined, and the tissue was transferred to scintillation counting vials. The samples were saponified with 0.75 M NaOH, scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes [33]. The rates of sugar uptake were determined as nanomoles of substrate absorbed per 100 mg dry weight of the mucosa per minute (Jm, nmol · 100 mg mucosal tissue^{-1} min^{-1}). Because the relationship between glucose uptake and concentration was curvilinear, the kinetic constants maximal transport rate (V_{max}) and Michaelis apparent affinity constant (Km) were calculated by nonlinear regression using the SigmaPlot program (Jandel Scientific, San Rafael, CA, USA). In addition, three linear transformations of the uptake data including the Lineweaver–Burk plot, the Wolfee plot and the Eadie–Hofstee plot were performed to confirm these kinetic estimates. Because fructose uptake was linear over the range of concentrations used (4–64 mM), the slopes of the

Table 1
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on jejunal and ileal morphology of postweanling rats

		Control	GLP-2	DEX	GLP-2+DEX
Jejunum	Villous height	149±49	212±4	219±15	203±9
	Villous width (base)	46±14	64±5	73±6	63±3
	Villous width (mid)	53±14	56±2	58±3	56±2
	Crypt depth	63±12	50±2	51±2	64±7
	Distance/5 villi	255±64	371±17	369±18	347±10
	Distance/5 cells	11±3	17±1	16±1	25±6
Ileum	Villous height	288±44	310±77	263±19	239±17
	Villous width (base)	117±14	116±33	105±7	113±5
	Villous width (mid)	113±21	105±37	95±1	95±5
	Crypt depth	88±12	87±29	88±7	104±13
	Distance/5 villi	893±170	838±153	670±32	703±45
	Distance/5 cells	42±7	31±4	26±1	27±2

Values are mean±S.E.M., $n=8$. Distant measurements are represented in micrometers.
None of these differences were statistically significant.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation.

Table 2
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on fructose uptake in postweanling rats

	Control	GLP-2	DEX	GLP-2+DEX
Jejunum	7.0±1.0 a	11.8±0.6 b	9.5±0.7 ab	11.6±1.3 b
Ileum	12.5±0.8 a	11.7±0.8 a	8.5±0.6 b	11.8±0.8 a

Values are mean±S.E.M., $n=8$.
Because fructose uptake is linear over the range of concentrations used (4–64 mM), the data is presented as slopes of the lines.
Values with different letters (a, b) are significantly different $P<.05$ by ANOVA.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation. The postweanlings were sacrificed on Day 49.

lines were calculated and compared to determine statistically significant differences between the treatment groups.

2.3. Morphological analysis

In order to determine the morphological characteristics of the intestine, a vertical section was prepared from the jejunum and from the ileum. Hematoxylin and eosin-stained slides were prepared from paraffin blocks. Crypt depth, villous height, villous width, villous width at half height, villous density and cell density were measured using the program MetaMorph 5.05r (Universal Imaging, Downingtown, PA, USA). The group means were obtained based on 10 villi and 20 crypts per slide, with a minimum of four animals in each group.

2.4. Immunohistochemistry

Jejunal and ileal tissues were embedded in paraffin, and 4–5-μm sections were mounted on glass slides. The sections were heated and placed immediately in xylene (2× for 5 min each), followed by absolute ethanol (2× for 2 min each), and were then rinsed with tap water. The slides were incubated in a hydrogen peroxide/methanol solution, and were then rinsed with tap water. Then, they were rehydrated, and the tissue was encircled on the slides with a hydrophobic slide marker (PAP pen, BioGenex, San Ramon, CA, USA). The slides were incubated for 15 min in blocking reagent (20% normal goat serum), followed by 30 min incubations with primary antibodies directed against sodium-dependent glucose transporter (SGLT1), GLUT2, GLUT5, α1 sodium potassium adenosine triphosphatase (Na^+K^+-ATPase), proliferating cell nuclear antigen (PCNA), PKA, PKC, phospho-p38, GSK-3, NOS3, phospho-Erk1/2, phospho-Akt1/PKBα and mammalian target of rapamycin (mTOR). All antibodies were obtained from Upstate Biotechnology (Lake Placid, NY, USA), with the exception of anti-SGLT1, anti- GLUT5 (Chemicon, Temecula, CA, USA), anti- GLUT2 (Biogenesis, Poole, England), anti-PCNA, anti-phospho-p38, anti-GSK-3 and anti-NOS3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). All antibodies were diluted 1:50 except for PCNA (1:200), SGLT1 (1:500), GLUT2 (1:500) and GLUT5 (1:250). The slides were incubated in LINK and LABEL and with DAB solution according to the manufacturers' protocol (BioGenex). The slides were then washed, stained in hematoxylin, dehydrated in absolute ethanol and cleared in xylene. The slides were photographed, and the area labeled with antibody was determined using Metamorph 5.05r. The results were expressed as a ratio of the area which was antibody-positive versus the total area. Statistical analyses were based on a minimum of four villi per animal and four animals per group.

Table 3
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on the V_{max} and Km for glucose uptake in postweanling rats

	V_{max}	Control	GLP-2	DEX	GLP-2+DEX
Jejunum	Sigmaplot	1624±316 a	1860±388 a	1472±129 a	3207±464 b
	Lineweaver–Burke	977±106 a	1079±93 a	1348±124 a	2033±120 b
	Wolfee	1590±166 a	1745±75 a	1490±106 a	2778±135 b
	Eadie–Hofstee	1115±121 a	1247±92 a	1393±117 a	2318±143 b
Ileum	Sigmaplot	1050±184 a	1658±143 a	1283±102 a	3286±972 b
	Lineweaver–Burke	719±77 a	1330±180 b	979±11 a	845±71 a
	Wolfee	1112±101 a	1629±190 b	1227±57 a	2155±165 c
	Eadie–Hofstee	789±88 a	1471±181 b	1111±31 ab	1141±80 ab
	Km	Control	GLP-2	DEX	GLP-2+DEX
Jejunum	Sigmaplot	22.2±10.2	27.4±12.6	21.4±4.5	50.9±13.2
	Lineweaver–Burke	5.9±0.0 a	7.7±0.4 b	18.5±0.4 c	23.1±1.6 d
	Wolfee	19.9±0.3 a	22.5±2.1 a	22.0±1.2 a	37.9±2.9 b
	Eadie–Hofstee	7.7±0.0 a	10.0±0.5 b	19.2±0.6 c	27.9±1.7 d
Ileum	Sigmaplot	13.9±6.8 a	29.0±5.4 a	36.9±5.8 a	118.9±49.2 b
	Lineweaver–Burke	4.5±0.4 a	19.6±1.2 b	23.2±3.4 b	15.3±1.2 b
	Wolfee	15.8±0.1 a	27.4±0.7 b	33.1±2.5 c	62.2±3.7 d
	Eadie–Hofstee	5.4±0.6 a	22.7±0.9 b	27.9±3.2 b	22.1±2.2 b

Values are mean±S.E.M., $n=8$.
Units: mM.
Values with different letters (a, b) are significantly different $P<.05$ by ANOVA.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation. The postweanlings were sacrificed on Day 49.

2.5. Apoptosis

Measurements of apoptosis were made based on cell morphology and assessed by a trained veterinary pathologist (R.R.E.U.) who was blinded to the treatment groups. Apoptotic cells were characterized with slight modifications of observations described by Potten et al. [34]. Apoptotic cells consisted of cells with intensely eosinophilic cytoplasm and nuclear chromatin that was either irregular and condensed or irregular and fragmented. The data are expressed as an "apoptotic index" in which the number of apoptotic cells is expressed as a percentage of the total epithelial cell numbers within the villus and the crypt.

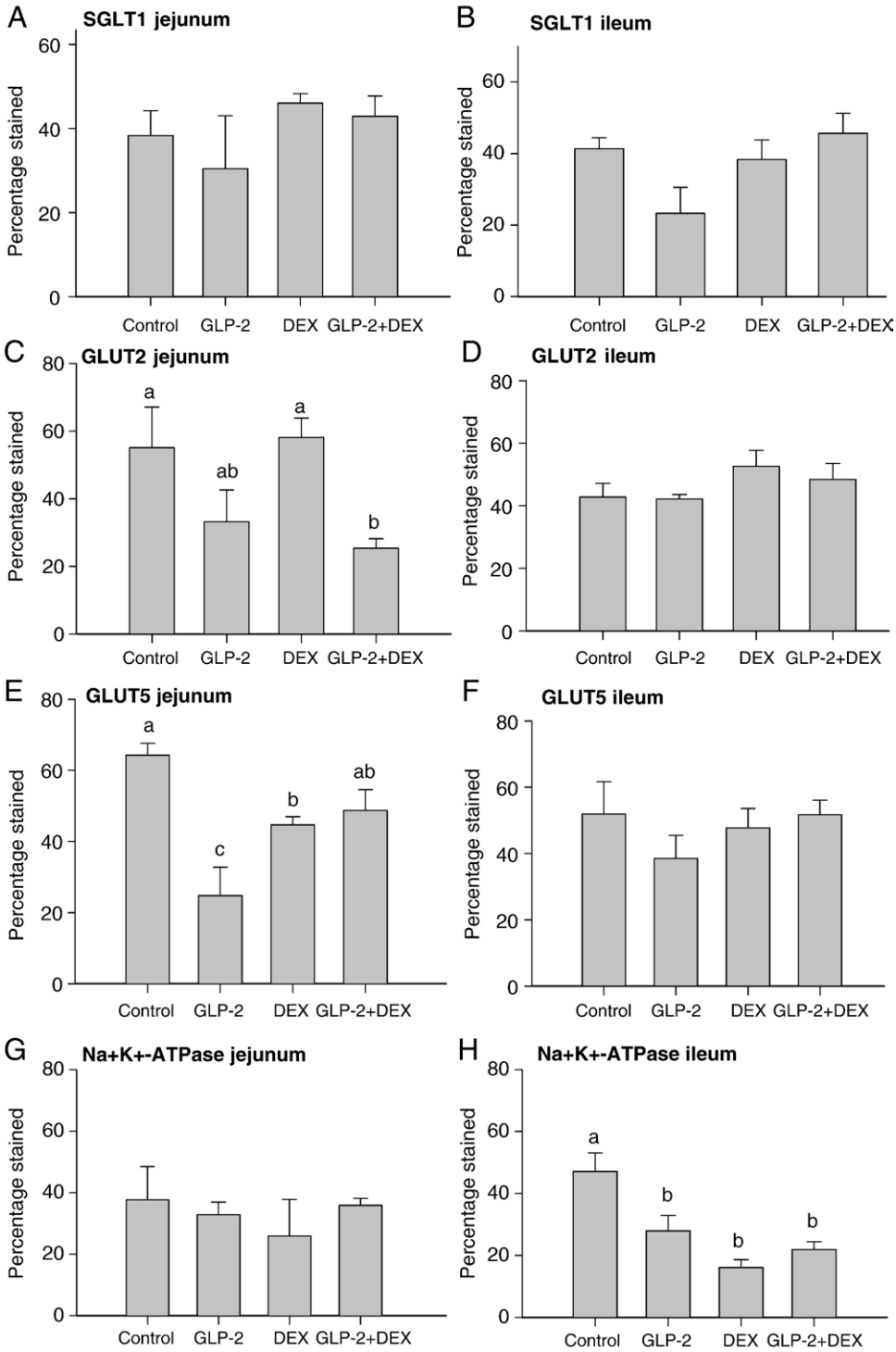

Fig. 2. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+ DEX on the abundance of SGLT1 (A,B), GLUT2 (C,D), GLUT5 (E,F), and Na^+K^+-ATPase (G,H) protein, as determined by immunohistochemistry.

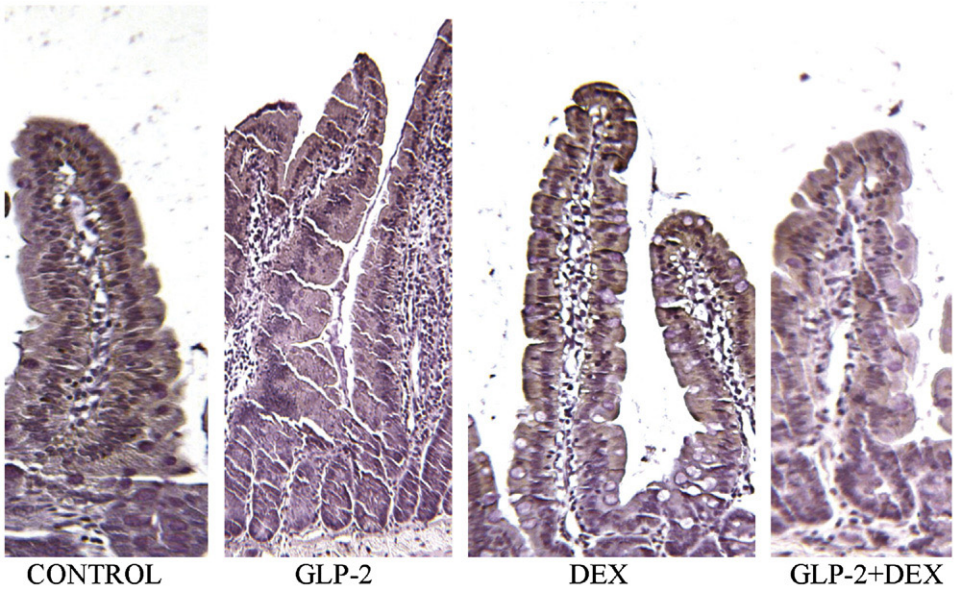

Fig. 3. Representative jejunal GLUT2 immunohistochemistry images.

2.6. Statistical analyses

The results were expressed as mean±S.E.M. The statistical significance of the differences between the four groups was determined by analysis of variance (ANOVA) and the Student Newman–Kuels test. Statistical significance was accepted for values of $P \leq .05$.

3. Results

3.1. Body and intestinal weights and villous morphology

There was no significant difference in the rate of body weight gain (g/day) among the dams in the control, GLP-2, DEX or GLP-2+DEX groups (data not shown). Also, there were no differences in the body weights of the postweanling rats in these four groups (data not shown).

In the dams, there was no effect of GLP-2, DEX or GLP-2+DEX on the weight of the jejunal or ileal mucosa, the weight of the intestine or on the percentage of the intestinal wall comprised of mucosa (data not shown). In the postweanling offspring, there was also no effect of GLP-2, DEX or GLP-2+DEX on the weight of the jejunal or ileal mucosa, the weight of the intestine or on the percentage of the intestinal wall comprised of mucosa (data not shown). Similarly, none of the treatments influenced the morphology of the postweanling intestine (Table 1).

3.2. Sugar uptake

Jejunal fructose uptake was increased by GLP-2 and by GLP-2+DEX, whereas ileal fructose uptake was reduced by DEX (Table 2). All four plots consistently showed that the value of the V_{max} for jejunal glucose uptake was increased by DEX+GLP-2 (Table 3). The value of the V_{max} in the ileum was also increased by GLP-2+DEX, and this was confirmed using the Wolfee plot (a similar trend was observed with the Lineweaver–Burke and Eadie–Hofstee plot, although the changes were not statistically significant). GLP-2 increased the value of the V_{max} for glucose uptake as well, although this was only statistically significant when linear transformations (Lineweaver–Burke, Wolfee and Eadie–Hofstee plots) were performed.

The value of the Km in the jejunum was increased by GLP-2+DEX, although this was only statistically significant when linear transformations (Lineweaver–Burke, Wolfee and Eadie–Hofstee plot) were performed. The Km in the ileum was increased by GLP-2+DEX, and this finding was confirmed by all three linear transformations (Lineweaver–Burke, Wolfee and Eadie–Hofstee plots). Both GLP-2 and DEX increased the value of the Km in the ileum, although this increase was only statistically significant when the linear transformations (Lineweaver–Burke, Wolfee and Eadie–Hofstee plot) were performed.

Table 4
The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+DEX on the apoptotic index in the jejunum and ileum of postweanling offspring

Apoptotic index	Control	GLP-2	DEX	GLP-2+DEX
Jejunum	2.2±0.2 a	0.7±0.1 b	0.3±0.1 b	0.2±0.1 b
Ileum	2.3±0.3 a	0.9±0.2 b	0.5±0.1 b	1.1±0.2 b

Values are mean±S.E.M., $n=2$–3.
Apoptotic index=no. of apoptotic cells/total number of cells×100.
Values with different letters (a, b) are significantly different $P<.05$ by ANOVA.
The treatments include GLP-2 (0.1 μg/g twice a day), DEX (0.128 μg/g once a day) and GLP-2+DEX at those doses, given during pregnancy and lactation. The postweanlings were sacrificed on Day 49.

3.3. Immunohistochemistry

3.3.1. Transporters

There was no change in the abundance of SGLT1 in the jejunum or ileum when comparing postweanling controls with GLP-2, DEX or GLP-2+DEX (Fig. 2A and B). GLP-2+DEX decreased GLUT2 abundance in the jejunum (Fig. 2C and Fig. 3), while none of the treatments significantly affected GLUT2 abundance in the ileum (Fig. 2D). GLP-2 and DEX decreased GLUT5 protein in the jejunum (Fig. 2E), while the treatments had no effect on the ileal GLUT5 abundance (Fig. 2F). While no changes were observed in the jejunal α1 Na^+K^+-ATPase abundance (Fig. 2G), each of the three treatments reduced ileal α1 Na^+K^+-ATPase (Fig. 2H).

3.3.2. Signals

GLP-2, DEX and GLP-2+DEX decreased apoptosis in the jejunum and ileum of postweanlings (Table 4). When the intestinal sections were divided into the upper villus, lower villus and crypt region, similar changes in the number of apoptotic cells were observed in all sections in response to the treatments (data not shown). All of the treatments increased PCNA abundance in the jejunum (Fig. 4A), while only DEX increased PCNA in the ileum, with GLP-2 and GLP-2+DEX decreasing the ileal abundance of PCNA (Fig. 4B).

There was no change in the jejunal or ileal abundance of PKA, PKC, MAPK, p38, GSK-3 or NOS3 when compared to control animals (data not shown). All three treatments increased protein kinase B (Akt) abundance in the jejunum

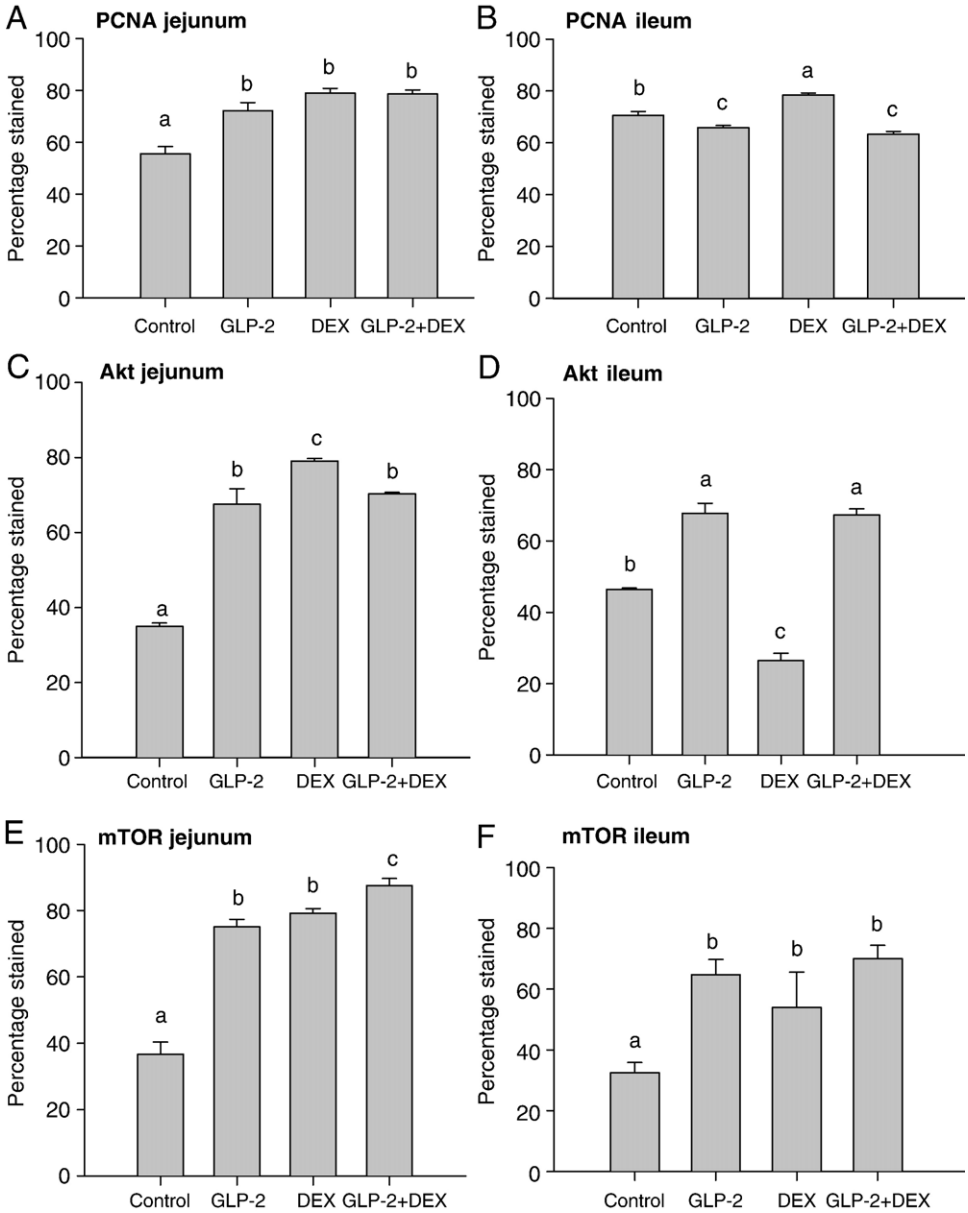

Fig. 4. The effect of treatment of pregnant and lactating rat dams with GLP-2, DEX or GLP-2+ DEX on the abundance of PCNA (A,B), Akt (C,D), and mTOR (E,F) protein, as determined by immunohistochemistry.

(Fig. 4C). GLP-2 and GLP-2+DEX increased Akt, and DEX decreased Akt in the ileum (Fig. 4D). All three treatments increased mTOR abundance in both the jejunum and ileum (Fig. 4E and F).

4. Discussion

In this study, the administration of GLP-2, DEX and GLP-2+DEX to rat dams and its effects on sugar uptake in their 4-week-old offspring was investigated. Measurements of sugar absorption, intestinal morphology and the expression of cellular proteins involved transport and signal transduction were considered.

There are several ways to estimate the value of the Km and the V_{max}, such as linear transformations of the Michaelis–Menton equation or the use of curve-fitting programs [35]. In this study, we used four methods to assess the effect of treatments on these kinetic parameters. Changes in intestinal sugar uptake are usually due to alterations in the value of the V_{max} rather than the Km [36,37]. However, in this study, both the V_{max} and the Km for ileal glucose uptake were increased by DEX+GLP-2 (Table 3). Alternatively, there may have been a change in the relative abundance of the various sugar transporters in the BBM. For example, glucose uptake across the BBM is mediated by SGLT1, but recent evidence suggests that under some experimental conditions, such as luminal glucose loading or high sugar diets, GLUT2 may traffic to the BBM and contribute to the enhanced uptake of both glucose and fructose [27,38–40]. Because SGLT1 has a low Km [41] and GLUT2 has a higher Km [42], an increase in BBM GLUT2 may explain the increased Km with GLP-2+DEX. This was not studied in this experiment but represents an interesting area for future work.

The combination of GLP-2+DEX resulted in the most pronounced increase in the value of the V_{max} for intestinal glucose uptake when compared to controls (Table 3). This suggests that the two agents may have an additive effect when given in combination. Interestingly, the combination of GLP-2+DEX did not have an additive effect on fructose uptake, with values for the combination being equivalent to those achieved with GLP-2 alone. This suggests that the mechanism by which the treatments altered glucose and fructose uptake may have differed.

Fructose and glucose uptake increased in the postweanling animals more than 1 month after treatment of their pregnant and lactating dams with GLP2+DEX (Tables 2 and 3). The mechanism of this late effect is unknown but may have important nutritional implications. Metabolic programming is a phenomenon that refers to the late effect of early environmental influences [4]. For example, epidemiological studies show that low birth weight is associated with increased risk of Type 2 diabetes and the metabolic syndrome [43]. Recently, Jacobson et al. [44] demonstrated that maternal dietary fat influences the composition of intestinal lipids and the responsiveness of the nursing offspring to experimental colitis.

Data from animal studies also show that manipulating the maternal diet has lasting effects on the intestinal uptake of nutrients in the offspring [1,6,8,11–13,43]. Thomson et al. [12] altered the ratio of polyunsaturated to saturated fatty acids in the diet of postweanling rats. They found that diets enriched in saturated fatty acids increased hexose uptake, and that these alterations were fast, progressive and irreversible. Feeding the same diets to pregnant and to lactating rats resulted in similar increases in sugar uptake in their postweanling offspring [8]. Curiously, these changes were not seen in suckling offspring, suggesting that the mechanisms responsible for adaptation may not be fully developed in these animals. This phenomenon was also observed in our studies. Increases in uptake were seen in the postweanling offspring of dams treated with DEX+GLP-2. However, these increases were not observed in the suckling (17–21 days old) offspring of the same dam (Drozdowski et al. 2006, unpublished work). This again suggests that the adaptive mechanisms are not fully functional in animals at this early age, but the effects of the stimulus of GLP-2 and DEX lasts into later life.

Increases in sugar uptake with GLP-2, DEX or GLP-2+DEX were observed in the jejunum and ileum of postweanling animals (Tables 2 and 3), despite there being no significant changes in ileal morphology. This, once again, highlights the complex relationship between intestinal morphology and intestinal transport and stresses that an alteration in intestinal morphology is not necessarily reflected by changes in absorption.

GLP-2 is trophic to the mature intestine [22], and the GLP-2 receptor of mature animals is most abundant in the proximal small intestine [45]. The GLP-2 receptor is present in the fetal and neonatal gut and responds to a degradation-resistant GLP-2 analog administered subcutaneously for 10 days [46]. Based on these observations, we expected to see increases in the intestinal villous height or crypt depth in GLP-2-treated animals. We did not see any significant effect of GLP-2 on intestinal morphology (Table 1), indicating that the route, dose and timing of our treatment did not produce a trophic effect on the postweanling intestine.

Increases were seen in jejunal PCNA abundance with GLP-2, DEX and GLP-2+DEX (Fig. 4A). However, glucose uptake was only increased with GLP-2+DEX, suggesting that there is not a direct relationship between proliferation and uptake. Assessment of jejunal apoptosis indicated that all three treatments significantly reduced the apoptotic index (Table 4). The reduction in apoptosis was most pronounced with GLP-2+DEX, and this reduction, coupled with the increase in proliferation, may be partially responsible for the increase in glucose uptake, possibly by altering the population of mature transporting enterocytes along the villus. Still, fructose uptake was increased to a similar extent by both GLP-2 and GLP-2+DEX, suggesting that factors other than proliferation and apoptosis are influencing intestinal transport.

While DEX can pass through the placenta and is present in milk [47], it is not known if the same is true for GLP-2. A study by Peterson et al. [48] demonstrated that the administering GLP-2 to fetal pigs in utero resulted in increased GLP-2 levels in the maternal circulation. However, whether or not maternal GLP-2 passes through the placenta and enters the fetal circulation remains to be proven. Several other growth factors and hormones are either produced by the placenta or have receptors on the placenta, such as insulin, IGF-1, IGF-II and EGF [49–54]. Similarly, many hormones and growth factors are present in breast milk [55]. Therefore, maternal GLP-2 may directly influence the offspring or may influence other growth factors or hormones that can pass through or from the placenta or into the breast milk and thereby indirectly alter intestinal morphology or sugar uptake in the suckling animals. Furthermore, the maternal administration of GLP-2 or DEX may alter the volume or composition of milk produced by the mother, which could potentially influence the offspring.

Immunohistochemistry (IHC) was used to assess the protein abundance of the sugar transporters SGLT1, GLUT2 and GLUT5. The abundance of the Na^+K^+-ATPase was also assessed, since it maintains the Na^+ gradient across the BBM, which is necessary for the optimal functioning of SGLT1. A correlation between IHC staining and protein levels, determined using other methods such as Western blotting [56–58] or immunoassays [59–62], has been shown. IHC has been used previously to demonstrate alterations in protein abundance in the intestine, such as with aging or as a result of modifications in dietary lipids [63–65]. GLUT2 is present in the basolateral membrane (BLM) and functions to transport glucose and fructose out of the enterocytes [66–68]. Under conditions of sugar loading, GLUT2 may traffic to the BBM to augment sugar uptake [27,38–40]. In these studies, IHC did not distinguish between the BBM or the BLM localization of GLUT2. In fact, GLUT2 in the jejunum was reduced with GLP-2+DEX, indicating that the IHC method used was sensitive enough to detect changes in protein abundance. In light of the reduction in sugar uptake, we speculate that the GLUT2 is largely intracellular and, therefore, nonfunctional. In the jejunum of postweanlings given GLP-2, GLUT2 fell but fructose uptake increased (Table 2, Fig. 2C). Thus, the transport alterations were not mirrored by changes in the abundance of GLUT2. Similarly, changes in sugar uptake were not explained by alterations in the abundance of SGLT1, GLUT5 or the Na^+K^+-ATPase.

The concept that there may be alterations in the intrinsic activity of a transporter is supported by the observation that there are discrepancies between glucose uptake and the protein abundance of glucose transporters in skeletal muscle [69], adipose tissue [70] and in the intestine [18,27,63,64,71–74]. In the intestine, changes in the intrinsic activity of glucose transporters have been observed with hyperglycemia [73], diabetes [72], low luminal glucose concentrations [40] and following the activation of MAPK and PI3K [27].

Although we selected several signaling proteins that may play a role in the regulation of intestinal sugar uptake [21,23,24,26,27,30,75–78], a number of these proteins (including PKA, PKC, MAPK, p38 and GSK-3) failed to demonstrate significant changes with GLP-2 or with DEX. However, both Akt and mTOR, members of the PI3K signaling pathway, were affected in this study. Helliwell et al. [27] showed that the PI3K pathway is involved in the modification of the intrinsic activity of GLUT2 and GLUT5. In this study, all three treatments significantly increased jejunal Akt abundance (Fig. 4C). Based on the results of Helliwell et al., one would expect an increase in Akt to produce an increase in GLUT2 and GLUT5 activity (fructose uptake), without increases in the abundance of these proteins. Indeed, when GLP-2+DEX were administered, the increase in jejunal Akt was associated with increased fructose uptake, while GLUT5 abundance was unchanged and GLUT2 abundance was actually decreased. Therefore, one may speculate that the changes in fructose uptake observed with GLP-2 and DEX are the result of PI3K-mediated alterations in the intrinsic activity of GLUT2 and GLUT5. Similarly, the increase in the uptake of glucose seen with DEX+GLP-2 in the jejunum of postweanlings was associated with increased abundance of Akt (Table 3, Fig. 4C). This supports the suggestion that these hormones alter the abundance and presumably the activity of Akt, which may, in turn, modify the intrinsic activity of BBM transporters. To investigate further the role of the PI3K pathway, we also determined the effect of DEX and GLP-2 on the abundance of mTOR, a downstream member of the PI3K pathway. In general, the changes seen in Akt (Fig. 4C and D) were mirrored by parallel alterations in mTOR (Fig. 4E and F), further implicating the PI3K pathway.

Modifications in the intrinsic activity of SGLT1 via the PI3K pathway have not been documented. However, a study by Alexander and Carey [25] showed that orogastric IGF-1 treatment increased glucose uptake in piglets without increasing SGLT1 abundance, suggesting an effect on the intrinsic activity of the transporter. This increase in uptake was blocked by a PI3K inhibitor, implicating this pathway in the IGF-1 induced changes in uptake. We speculate that a similar mechanism may provide an explanation for the variations in glucose uptake observed in this study in response to DEX+GLP-2.

Increases in jejunal Akt and mTOR (Fig. 4C and E) were associated with an enhancement in intestinal proliferation (Fig. 4A) and a decrease in apoptosis (Table 4). Akt inhibits apoptosis by phosphorylating the proapoptotic protein Bad and by blocking cytochrome *c* release [79]. mTOR may also regulate apoptosis as a member of an apoptotic pathway, activated by microtubule damage and inducing phosphorylation of Bcl2 [80]. Furthermore, the PI3K/Akt pathway is thought to transduce proliferative signals from growth factor receptors to the cell cycle

machinery of intestinal epithelial cells. Indeed, Akt increases cyclin D expression, promoting entry into the S phase [81], while mTOR regulates several cell cycle proteins including pRb and $p27^{KIP1}$ [80]. Our data support the view that increases in Akt and mTOR are associated with increases in proliferation and decreases in apoptosis in the intestinal epithelial cells.

In conclusion, the maternal administration of GLP-2+DEX increases intestinal sugar uptake in their postweanling offspring. The mechanism of this late effect is unknown, but associated increases in Akt and mTOR suggest that alterations in the intrinsic activity of the sugar transporters may play a role. This study supports the concept that early exposure to maternal factors influences physiological functioning later in life and focuses attention on the importance of the fetal and neonatal environment.

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