J Appl Physiol Articles in PresS. Published on September 13, 2002 as DOI 10.1152/japplphysiol.00134.2002

LOCALLY AND SYSTEMICALLY ACTIVE GLUCOCORTICOSTEROIDS MODIFY INTESTINAL ABSORPTION OF SUGARS IN RATS

A. Thiesen^, G.E. Wild[#], M. Keelan^, M.T. Clandinin* and A.B.R. Thomson^

Cell and Molecular Biology Collaborative Network in Gastrointestinal Physiology

^Nutrition and Metabolism Research Group
Division of Gastroenterology
Department of Medicine
University of Alberta
Edmonton, Canada,

*Department of Food Sciences and Nutrition
University of Alberta
Edmonton, Canada,

and

[#]Division of Gastroenterology , and
Department of Anatomy and Cell Biology
McGill University
Montreal, Quebec
Canada

Short Title: Steroids and Gut Absorption

Index Words: adaptation, budesonide, GLUT5, prednisone, SGLT1

Submitted to Journal of Applied Physiology

2002

Please send correspondence to:

Alan B.R. Thomson
519 Robert Newton Research Building
University of Alberta
Edmonton, Alberta, Canada T6G 2C2
Telephone: (780) 407-6490
Fax: (780) 407-7964
e-mail: alan.thomson@ualberta.ca

ABSTRACT

Glucocorticosteroids enhance digestive and absorptive functions of the intestine of weaning and adult rats. This study was undertaken to assess the influence of treatment of weaning male rats with budesonide, prednisone, or control vehicle (CON) on the *in vitro* jejunal and ileal uptake of glucose and fructose. BUD and PRED had no effect on the uptake of D-glucose by SGLT1. In contrast, the uptake of D-fructose by GLUT5 was similarly increased with BUD and with PRED. The increases in the uptake of fructose were not due to variations in the weight of the intestinal mucosa, food intake, or in GLUT5 protein or mRNA expression. There were no steroid-associated changes in mRNA expression of c-myc, c-jun, c-fos, of proglucagon, or of selected cytokines. However, the abundance of ileal ODC mRNA was increased with PRED. Giving post- weaning rats four weeks of BUD or PRED in doses equivalent to those used in clinical practice increases fructose but not glucose uptake. This enhanced uptake of fructose was likely regulated by post-translational processes.

Key Words: adaptation, budesonide, GLUT5, prednisone, SGLT1

ABBREVIATIONS

BUD	budesonide
cDNA	complimentary DNA
DNA	deoxynucleic acid
EDTA	ethylenediamine-tetraacetic acid
L-FABP	liver fatty acid binding protein
ILBP	ileal lipid binding protein
mRNA	messenger RNA
PRED	prednisone
RNA	ribonucleic acid
SD	standard deviation
SEM	standard error of the mean
UV	ultraviolet

INTRODUCTION

Glucocorticosteroids ("steroids") are widely used to treat a variety of gastrointestinal and hepatic conditions, such as inflammatory bowel diseases and chronic active hepatitis (1-6). However, the systemically active steroids may be associated with potentially serious adverse effects (1,7-9). The high prevalence of these adverse effects has been a major impetus for the development of non-systemic steroids. Budesonide is a non-systemic steroid with high topical activity, low systemic bioavailability, and rapid first pass metabolism in the intestine and liver (1,10). Budesonide is of proven clinical efficacy when given topically or orally to patients with inflammatory bowel disease (11,12).

In young animals steroids induce precocious development of some of the intestinal brush border membrane (BBM) enzymes, and facilitate the induction of specific enzymes by dietary carbohydrate (13-15). Systemically active steroids given by mouth enhance glucose uptake by adult animals (16). Dexamethasone (128 μg/kg/day) given subcutaneously for seven days blunts the expected adaptive response following intestinal resection (17).

The Na^+ -gradient across the BBM provides the driving force for glucose transport (18). This gradient is maintained by the action of the Na^+/K^+-ATPase, which is restricted to the basolateral membrane of the enterocyte (19). SGLT1 mediates the BBM Na^+/glucose cotransport (20-22). Fructose uptake across the BBM is mediated by facilitated diffusion by GLUT5 (23-26), whereas GLUT2 mediates the facilitative Na^+-independent diffusion of glucose and fructose through the BLM (27). Recent evidence suggests that GLUT2 may also be in the BBM (28-30).

Proglucagon-derived peptides originate from processing and breakage of the proglucagon gene product (31,32) in the L-cells present in the ileum and colon (33). Ornithine decarboxylase (ODC) is a key enzyme in the synthesis of polyamines, a requirement for any proliferative event. Early response genes (ERG) are genes expressed in response to proliferative stimulation. It has been suggested that the mRNA levels of proglucagon and ornithine decarboxylase as well as the mRNAs of early response genes such as c-myc, c-jun and c-fos may be involved in the intestinal adaptive process such as resection of the small intestine (34-37). It is unknown if proglucagon, ODC or ERGs in the intestine are influenced by steroids.

A wide variety of cytokines are produced locally by the intestinal epithelium, and they are involved in the homeostasis of the intestinal tissue during development (38,39). Cytokine gene expression has been shown to be regulated by hydrocortisone and dexamethasone during postnatal small intestinal development (40). Cytokines alter sugar absorption (41-43). It is not known if the cytokines in the intestine are influenced by prednisone or budesonide.

Accordingly, this study was undertaken to assess the influence of budesonide and prednisone, in doses equivalent to those used in clinical practice, on 1) the intestinal uptake of glucose and fructose in young growing rats; 2) the abundance of the glucose and fructose transporter proteins and the expression of their respective mRNAs; and 3) the mRNA expression of several potential signals of steroid-associated intestinal adaptation including proglucagon, ODC, three ERGs (c-myc, c-fos and c-jun), and selected cytokines (TNF-α, IL-2, IL-6 and IL-10).

METHODS AND MATERIALS

Animals and drugs

The principles for the care and use of laboratory animals approved by the Canadian Council on Animal Care and the Council of the American Physiological Society, were observed in the conduct of this study. Weanling male Sprague Dawley rats, 21-23 days of age *post-partum*, were obtained from the University of Alberta Vivarium. Pairs of rats were housed at a temperature of 21 ^{0}C, with 12 hours of light and 12 hours of darkness. Water and food were supplied *ad libitum.* The animals were fed standard Purina^R rat chow.

It was previously calculated with a realibility coefficient of 95 %, a margin of error of 3 and a population standard error (estimation) of 18 that a sample size of 8 would be statistically sufficient to detect the differences if they were present. For the dosing study a sample size of 6 was determined to be sufficient in order to detect any major differences between the groups. For the gene expression studies, we used a sample size of 3, which allowed us to make preliminary observations. Therefore, negative results should be carefull considered and not discarded, because the sample size may not have been large enough to demonstrate true differences.

There were 8 animals in each of 3 groups: 1) control (0.19% EDTA buffered saline), 2) budesonide (0.25 mg/kg body weight per day), and 3) prednisone group (0.75 mg/kg body weight per day). The doses of prednisone and budesonide were chosen on the basis of regimens which

have been shown to be useful clinically in humans (7,44,45). The steroids were administrated by oral gavage each day, and were dissolved in 0.19% EDTA buffered saline. The volume of vehicle given was 5 µl/g body weight. Animals were gavaged for a duration of 4 weeks with drug or vehicle at 12:00 h daily, including weekends. This range is similar to the doses (0.2-0.8 mg/kg/day) used to treat trinitrobenzene sulphonic acid ileitis in rats (44), and the doses (0.1-1.0 mg/kg day) used to prevent graft rejection in a rat model of intestinal transplantation (45).

A dosing study with budesonide was also performed. There were 6 animals in each group, and the doses of budesonide which were used were 0.50, 0.75 and 1.00 mg/kg day for 2 weeks.

Probe and marker compounds

The [^{14}C]-labelled probes included L-glucose (16 mM), D-mannitol (16 mM), and a range of concentrations of D-glucose and D-fructose (4, 8, 16, 32 and 64 mM). Unlabelled and [^{14}C]-labelled probes were supplied by Sigma Co. (St Louis, Missouri) and by New England Nuclear, respectively. [^{3}H]-inulin was used as a non-absorbable marker to correct for the adherent mucosal fluid volume. Probes were shown by the manufacturer to be more than 99% pure by high performance liquid chromatography.

Tissue preparation and determination of uptake rates

The animals were sacrificed by the injection of Euthanyl^{R} (sodium pentobarbitol, 240 mg/100 g body weight). The whole length of the small intestine was rapidly removed. The proximal third beginning at the ligament of Treitz was termed the jejunum, and the distal third was termed the ileum; the middle third of the small intestine was discarded. The intestine was everted and cut into small rings of length approximately 2-4 mm each (46). The rings were immersed immediately in preincubation beakers containing oxygenated Krebs-bicarbonate buffer (pH 7.2) at 37 ^{0}C, and were allowed to equilibrate for approximately 5 min prior to commencement of the uptake studies. Uptake was initiated by the timed transfer of tissue rings to a shaking water bath (37 ^{0}C) containing 5 ml plastic vials with gassed Krebs buffer (95% O_2, 9% CO_2), plus [^{3}H]-inulin and [^{14} C]-labelled substrates. After incubation for 5 min, the uptake of nutrient was terminated by pouring the vial contents onto filters immobilized on an Amicon vacuum filtration manifold maintained under suction. This was followed by washing the jejunal or ileal rings with ice-cold

saline. The tissue was dried, and the weight was recorded prior to saponification of the tissue with 0.75 N 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.

The rates of uptake of the sugars were expressed as nmol of substrate taken up per 100 mg dry weight of intestinal tissue per minute (nmol*100 mg tissue^{-1}*min^{-1}). The values obtained from the three treatment groups (control vehicle, budesonide and prednisone) were reported as the mean ± SEM of results obtained from 8 animals in each group.

The values of the maximal transport rate (Vmax) and the apparent Michaelis constant (Km) for glucose uptake were estimated using non-linear regression (Sigma Plot program, Jandel Scientific, San Rafael, CA). Because the relationship between fructose concentration and uptake was linear over the range of concentrations studied (4-64 mM), the values of Vmax and Km could not be calculated. Instead, linear regression was used to obtain the value of the slope of this linear relationship.

Membrane preparation

There were 8 animals in each of the 3 drug groups (control, budesonide and prednisone). Two 40 cm lengths of proximal jejunum and distal ileum were rapidly removed and rinsed gently with ice-cold saline. The intestine was opened along its mesenteric border, and the mucosal surface was washed carefully with cold saline to remove mucus and debris. The mucosal surface was blotted with lint-free tissue to remove excess moisture, and was removed from the rest of the intestinal wall by gently scraping with a microscopic slide and then snap-freezing the tissue in liquid nitrogen at -80°C for later membrane preparation. Brush border membranes (BBM) and basolateral membranes (BLM) were isolated from the rat intestinal mucosal scrapings using homogenization, differential centrifugation, and Ca^{2+} precipitation (47-49). Aliquots were stored at -80°C for Western immunoblotting.

Western immunoblotting

BLM and BBM proteins were separated by SDS-PAGE (sodium docecyl sulfate-polyacrylamide gel electrophoresis). After electrophoresis, proteins were immobilized and transferred to nitrocellulose by electroblotting. Then, the membranes were blocked by incubation overnight in 5% w/v dry milk in Tween Tris Buffered Saline (TTBS: 0.5% Tween 20, 30mM Tris,

150 mM NaCl). Membranes were subsequently washed three times with TTBS and probed with specific rabbit anti-rat antibodies: α1-Na^+/K^+ ATPase, β1-Na^+/K^+ ATPase, GLUT2, GLUT5 and SGLT1. The antibodies were diluted in 2% dry milk in TTBS and the incubations wetre done at room temperature.

The polyclonal antibodies against SGLT1 and GLUT2 were obtained from Biogenesis, Poole, England. The polyclonal antibody against GLUT5 was obtained from Chemicon International Inc., Temecula, California. The polyclonal antibodies anti-rat α1 and β1-Na^+/K^+ ATPase were obtained from Upstate Biotechnology Inc., Lake Placid, NY.

Following incubation in primary antibody, membranes were washed three times with TTBS. Membranes were then incubated with goat anti-rabbit antibody conjugated with horseradish peroxidase, HRP, (Pierce, Rockfort, Illinois, USA). After three washes in TTBS, the immune complexes were visualized with SuperSignal® Chemiluminescent-HRP Substrate (Pierce, Rockfort, Illinois, USA). Agter exposure to X-OMAT AR film, the relative band densities were determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life science group, Cleveland, Ohio, USA).

Northern Immunoblotting

Complementary DNA (cDNA) probes were produced. Bacteria (*E. coli*) were transformed with plasmids containing the desired DNA sequences to be probed for the Northern blotting. SGLT1 cDNA probe was donated by Dr. Davidson, University of Chicago; cDNA probes encoding the α1 and β1 Na^+/K^+ ATPase subunit isoforms were obtained from Dr. Lingrel, University of Cincinnati; cDNA probes encoding GLUT5 and GLUT2 were obtained from Dr. Bell, University of Chicago; ERG probes were obtained from Oncogene Research Products; cDNA probe encoding proglucagon was obtained form Dr. Fuller, Prince Henry's Institute of Medical Research in Melbourne; ODC was obtained from Dr. Blackshear, University of Chicago; and TNF-α, IL-2, IL-6 and IL-10 were obtained from BIO/CAN Scientific. A DIG labelled nucleotide (Roche Diagnostics, Quebec, CA) was incorporated during the DNA synthesis using a DNA polymerase (Roche Diagnostics, Quebec, CA). The probe concentration was estimated according to comparison with the intensity of a control pre-labelled DNA (Roche Diagnostics, Quebec, CA).

RNA was extracted from the mucosal scrapings of the jejunum and ileum obtained from at least three animals in the 3 groups. These intestinal segments were homogenized in a denaturing solution containing guanidinium thiocyanate, using a biorad fast prep shaking centrifuge. Following addition of 2M sodium acetate, a phenol chloroform extraction was performed. The upper aqueous phase was transferred to a tube, and the RNA was precipitated with isopropanol and washed with 70% ethanol. RNA samples were stored at -70°C.

Total RNA was electrophoresed through a denaturing agarose gel (1.16% agarose) and then transferred from the gel to a nylon membrane by capillary action, overnight. Membranes were then baked at 80°C for 2 hours to fix the RNA onto the membrane. As a pre-hybridization, membranes were incubated for 30 minutes with DIG easy hyb solution (Roche Diagnostics, Quebec, CA). Following pre-hybridization, the labelled probes were hybridized to the corresponding RNA band on the membrane by incubation with the DIG labelled probe at the adequate temperature overnight. After stringency washes, membranes were blocked in 1 x blocking solution (10% 10 x blocking solution, 90% 1 x maleic acid). The membranes were then incubated with an anti-DIG-AP conjugate antibody (Roche Diagnostics, Quebec, CA).

The detection of the bound antibody was performed using a CDP-STAR chemiluminescent substrate (Roche Diagnostics, Quebec, CA), and membranes were exposed to films (X-Omat, Kodak, USA) for 10-30 minutes. The density of the RNA was determined by transmittance densitometry using a Bio-Rad imaging densitometer (Life Science Group, Cleveland, Ohio, USA).

RESULTS

Animal Characteristics

Food intake was similar in the control vehicle, budesonide- and prednisone - treated animals (Table 1). Despite this, weight gain was lower ($p < 0.05$) in the budesonide than in the prednisone or in the control group. The body weight gain in rats given budesonide 0.75 and 1.0 mg/kg was similar to controls (data not shown). The percentage of weight gain (g/day) per food intake (g/day) was lower in the budesonide than in the control group, was higher in the prednisone than in the control or budesonide group.

The mean weight of the intestine (mg/cm length) and the percentage of the intestinal wall comprised of mucosa were similar in the control, prednisone and budesonide groups (Table 2).

Accordingly, the rates of sugar uptake were expressed as nmol of substrate taken up per 100 mg dry weight of intestinal tissue per minute (nmol*100 mg tissue^{-1}*min^{-1}).

Uptake of Sugars

A curvilinear relationship was noted between the concentration of D-glucose (4-64 mM) and the rate of glucose uptake (Figure 1). The estimated values of the maximal transport rate (Vmax) and of the apparent Michaelis constant (Km) for glucose uptake were unaffected by treatment with prednisone or with budesonide (0.25 mg/kg) (Table 3). Budesonide given at a dose of 1.0 mg/kg also had no effect on D-glucose uptake (data not shown). The jejunal and ileal rates of uptake of L-glucose and of D-mannitol were unaffected by prednisone or budesonide, as compared with the control group (Table 4).

A linear relationship was noted between increasing concentrations (4-64 mM) and the rate of uptake of fructose (Figure 2). Because this relationship was linear over the concentrations studied, it was not possible to calculate values for Vmax or for Km. In the jejunum and ileum, the value of the slope of this linear relationship was higher ($p<0.05$) in the prednisone and in the budesonide groups, as compared with the control group (Table 5). A dose of budesonide of 1 mg/kg also increased ($p<0.05$) the slope of fructose uptake into the jejunum to 13.9 nmol*100mg tissue^{-1}*min*mM^{-1}, as compared with 12.1 nmol*100 mg tissue^{-1}*min*mM^{-1} in the control group (Table 5).

Transporter Protein Abundance and Expression of mRNA

In animals given prednisone the SGLT1 abundance was reduced ($p<0.05$) in the jejunum as compared with the control group, and did not change in the ileum (Table 6). Budesonide did not affect the abundance of SGLT1. The Na^+/K^+-ATPase alpha 1 was not changed in either the jejunum or ileum (Table 6). No changes in Na^+/K^+-ATPase beta 1 abundance were observed in the jejunum. However, the Na^+/K^+-ATPase beta 1 abundance was reduced in the ileum of animals given prednisone, as compared to animals given control vehicle or given budesonide. Steroids had no effect on GLUT5 and GLUT2 abundance in the jejunum or ileum.

No differences in mRNA expression of SGLT1, Na^+/K^+-ATPase alpha 1 or Na^+/K^+-ATPase beta 1 were observed in the jejunum and ileum of animals given budesonide or

prednisone, as compared with the control group (Table 7). Steroids had no effect on GLUT5 or GLUT2 mRNA expression in the jejunum or ileum.

Early Response Gene, Proglucagon and Ornithine Decarboxylase (ODC) mRNA Expression

No detectable signal was observed for c-fos. Steroids had no effect on the expression of c-myc and c-jun (Table 8).. Steroids had no effect on proglucagon mRNA expression at either site. In the ileum but not in the jejunum of animals given prednisone, the ODC mRNA expression was increased as compared to animals in the control group or those given budesonide.

Cytokine Gene Expression

Steroids had no effect on the mRNA expression of TNF-a, IL-2, IL-6 or IL-10 in either the jejunum or ileum (Table 9).

DISCUSSION

Animals fed budesonide had a reduced rate of weight gain which was not explained by a lower food intake, or by a lower rate of intestinal uptake of glucose or fructose. In fact, animals given budesonide had increased uptake of fructose (Table 5). The mechanism responsible for this lower weight gain in rats given budesonide was not established in this study. However, it is possible that the effect of budesonide on weight gain may have been spurious, firstly because at higher doses (0.75 and 1.0 mg/kg) weight gain was similar to controls, and secondly because budesonide (0.25 mg/kg) had no effect on body weight gain in animals fed a semisynthetic diet enriched with saturated or polyunsaturated fatty acids (unpublished observations, 2001). It is interesting to note that the dose of prednisone used in this study did not alter food intake or body weight gain, despite its systemic nature.

Prednisone acts systemically on the intestine, in contrast to the largely local action of budesonide (1). In adult animals, prednisone (in a dose of 0.75 mg/kg for 28 days) increases glucose absorption (16). The lack of effect of prednisone on glucose uptake in this study may be due to the younger age of the animals. Other functions of the intestine have been shown to change with the administration of steroids and the time or age of the animals is critical. For instance, starting dexamethasone on the 16^{th} day produces an accelerated rise in sucrase-isomaltase (SI) and SI mRNA, but starting on 18^{th} day did not have an effect (50). The lack of effect of prednisone or budesonide on the jejunal or ileal uptake of L-glucose or D-mannitol

(Table 4) suggests that the passive paracellular contribution to sugar uptake is also unaffected by these steroids. The lack of effect of either prednisone or budesonide on the value of the Vmax of glucose uptake in these four week post-weanling rats (Table 3) suggests that there was no change in the activity of the sodium-dependent glucose transporter in the brush border membrane, SGLT1. The reduced jejunal abundance on SGLT1 in animals given prednisone (Table 6) did not affect the activity of the transporter. This suggests that under some conditions there may be a dissociation between SGLT1 protein abundance and transporter activity.

The linear relationship between fructose uptake and concentration (over the range used in this study) precluded the calculation of values for Vmax or Km. Fructose uptake is mediated by GLUT5 (the sodium-independent fructose transporter in the brush border membrane). In this study the increased fructose uptake with budesonide or prednisone was not associated with enhancement in the abundance of GLUT5 protein or expression of GLUT5 mRNA. This suggests that the increase in fructose uptake observed with steroids is due to post-translational control of GLUT5. Another possibility would involve the distribution of GLUT5 along the crypt-villus unit that could be altered without changes in the total abundance of GLUT5, as measured by Western blotting (51). GLUT2 transports fructose across the basolateral membrane, and recent evidence suggests that GLUT2 may also be in the BBM (28-30). However, no changes in GLUT2 protein abundance or mRNA expression were observed, so that it is unlikely that the increased fructose uptake observed with steroids could be explained by enhanced transport of this sugar out of the enterocyte.

Steroids have been suggested to increase the expression of a series of transcription factors (52-55). Early response genes such as c-myc, c-jun and c-fos have been demonstrated to be involved in processes of proliferation and differentiation, as well as ODC, a key enzyme in the synthesis of polyamines and a requirement in any proliferative event (34-37). Proglucagon has been shown to be involved in the intestinal adaptive process (31,32,37). For example, short chain fatty acids increase the ileal c-myc and proglucagon expression in rats undergoing intestinal resection (37). The finding in this study of increased ileal ODC mRNA with prednisone (Table 8) may explain part of the enhanced fructose uptake with this steroid, but does not explain the enhancing effects of budesonide on fructose uptake. ODC, may be responsible for the increased

uptake of D-fructose in animals given prednisone. By a mechanism probably involving proliferative events, ODC might be able to induce transporters such as GLUT5 and consequently absorption. Clearly, there must be other signals responsible for the adaptive effect of steroids on intestinal fructose uptake.

The major factor for the understanding of the absence of changes in the expression of ERG comes from the fact that major changes observed in previous studies occur in the first 24 hours after the stimuli (37). We have looked only after two weeks of steroid administration, since we wanted to assess the chronic effect of steroids on ERG expression. Therefore, initial increases in ERG may in fact have occurred, and ERG may be of physiological and pathological importance at the early stage after the administration of steroids, rather than after later administration, as was the case in this study.

The administration of IL-6, IL-1α and IL-8 has been show to increase the uptake of glucose *in vitro* studies (42). It was hypothesized that changes in cytokine expression might be responsible for the phenotypic alterations in transport activity and absorption acting by intracellular signalling mechanisms that would result in expression of transporters (41-43). However, cytokine signalling was not observed with either prednisone or budesonide. Therefore, the effect of steroids on the fructose uptake was not explained by alterations in the mRNA expression of TNF-α, IL-2, IL-6 and IL-10.

In summary, 1) giving post- weaning rats four weeks of budesonide or prednisone in doses equivalent to those used in clinical practice increases fructose but not glucose uptake; and 2) the enhanced uptake of fructose was likely regulated by post-transcriptional events.

ACKNOWLEDGEMENTS

The authors wish to thank Kim Doring, Rob Drummond, and Elizabeth Wierzbicki for their technical assistance. The financial support of the National Sciences and Engineering Research Council of Canada, Medical Research Council (MRC) of Canada, and of AstraZeneca Canada Inc. (former Astra Pharma Inc.) with an MRC/PMAC grant, was greatly appreciated. We also wish to thank Ciencia Laboratorio Medico and CAPES for the support of Dr. Aducio Thiesen. Dr. G. Wild is a research scholar of Les Fonds de la Recherche en Sante du Quebec.

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TABLES AND FIGURES

Table 1: Food Intake and Body Weight Gain.

	Food Intake (g/day)	Weight Gain (g/day)	Weight Gain per Food Intake, %
Control Vehicle	22.3 ± 0.8	9.1 ± 0.2	40.8 ± 0.4
Prednisone	21.1 ± 1.1	9.0 ± 0.2	42.6 ± 0.7*
Budesonide	21.0 ± 0.8	8.2 ± 0.3*	39.0 ± 0.4*#

Mean ± SEM. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$, budesonide or prednisone vs control vehicle.
#, $p<0.05$, budesonide vs prednisone.

Table 2: Characteristics of Intestine.

	Intestinal Weight (mg/cm)	% of Intestinal Wall Comprised of Mucosa
Jejunum		
Control Vehicle	20.0 ± 0.7	53.4 ± 3.2
Prednisone	22.6 ± 2.1	45.7 ± 6.0
Budesonide	18.3 ± 1.2	53.3 ± 3.6
Ileum		
Control Vehicle	15.4 ± 2.8	47.6 ± 6.1
Prednisone	13.5 ± 1.1	37.5 ± 6.2
Budesonide	10.7 ± 0.7	38.9 ± 6.4

Mean ± SEM. The dose of budesonide was 0.25 mg/kg, and the prednisone dose was 0.75 mg/kg.

Values were not significantly different.

Table 3: Kinetic Constants of Intestinal Uptake of D-Glucose.

	Maximal Transport Rate Vmax (nmol.100 mg-1.min-1)	Apparent Michaelis Constant Km (mM)
Jejunum		
Control Vehicle	1492 ± 84	47 ± 5
Prednisone	1366 ± 150	43 ± 9
Budesonide	1468 ± 58	44 ± 3
Ileum		
Control Vehicle	1652 ± 272	80 ± 21
Prednisone	1659 ± 382	85 ± 30
Budesonide	1808 ± 373	82 ± 26

Mean ± SEM. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

Values were not significantly different.

Table 4: Rates of Uptake of L-glucose and D-mannitol.

	L-Glucose	D-Mannitol
Jejunum		
Control Vehicle	14 ± 1	17 ± 1
Prednisone	16 ± 2	18 ± 1
Budesonide	14 ± 1	16 ± 1
Ileum		
Control Vehicle	10 ± 1	12 ± 1
Prednisone	10 ± 1	14 ± 2
Budesonide	10 ± 1	13 ± 1

Mean ± SEM. The rates of uptake are expressed as nmol*100 mg tissue-1 *mM-1· mM^{-1}. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

Values were not significantly different.

Table 5: Slopes of Relationship Between Concentration and Rates of Uptake of D-Fructose in the Jejunum and Ileum

A. Steroid Study

	Jejunum	Ileum
	Slope	Slope
Control Vehicle	12.1 ± 0.3	10.1 ± 0.3
Prednisone	13.2 ± 0.1*	11.7 ± 0.2*
Budesonide (0.25 mg/kg)	12.8 ± 0.1*	12.1 ± 0.8*

B. Dosing Study

	Jejunum	Ileum
	Slope	Slope
Control Vehicle	12.6 ± 1.3	10.7 ± 0.8
Budesonide (0.50 mg/kg)	15.8 ± 0.2*	9.9 ± 0.6
Budesonide (0.75 mg/kg)	17.0 ± 0.4*	8.6 ± 0.6 ~
Budesonide (1.0 mg/kg)	13.9 ± 0.4 @	10.7 ± 0.8

Mean ± SEM. The values of the slopes are expressed as nmol * 100 mg tissue-1 * min-1*. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

*, $p<0.05$, budesonide or prednisone vs control vehicle.

@, $p<0.05$, budesonide (1.0mg/kg) vs budesonide (0.75 mg/kg)

~, $p<0.05$, budesonide (1.0mg/kg) vs budesonide (0.50 mg/kg).

Table 6: Protein Abundance Related to D-glucose and Fructose Uptake.

	SGLT1	Na^+/K^+ ATPase alpha1	Na^+/K^+ ATPase Beta 1	GLUT5	GLUT2
Jejunum					
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	0.47 ± 0.29*	1.13 ± 0.14	1.13 ± 0.13	1.13 ± 0.75	1.02 ± 0.31
Budesonide	0.86 ± 0.53	1.24 ± 0.40	1.00 ± 0.27	0.93 ± 0.37	1.09 ± 0.12
Ileum					
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.16 ± 0.36	1.53 ± 0.37	0.73 ± 0.33*#	0.98 ± 0.33	1.30 ± 0.55
Budesonide	0.86 ± 0.35	0.94 ± 0.63	0.99 ± 0.24	0.91 ± 0.37	1.39 ± 0.48

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.
P = Prednisone.
B = Budesonide.
C = Control.

*, $p<0.05$, prednisone vs control vehicle.
#, $p<0.05$, budesonide vs prednisone.

Table 7: mRNA Expression Related to D-glucose and Fructose Uptake.

	SGLT1	Na^+/K^+ ATPase alpha1	Na^+/K^+ ATPase beta1	GLUT5	GLUT2
Jejunum					
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	0.69 ± 0.69	0.79 ± 0.42	0.57 ± 0.42	0.83 ± 0.28	1.01 ± 0.26
Budesonide	1.21 ± 0.48	2.15 ± 1.81	1.23 ± 0.87	0.83 ± 0.44	1.23 ± 0.65
Ileum					
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.47 ± 0.45	1.20 ± 0.59	1.41 ± 0.75	1.41 ± 0.41	1.62± 0.93
Budesonide	1.32 ± 0.55	1.29 ± 0.41	2.58 ± 3.41	0.56 ± 0.44	1.10 ± 0.27

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.
P = Prednisone.
B = Budesonide.
C = Control.

Values were not significantly different.

Table 8: mRNA Expression of Early Response Genes, Ornithine Decarboxylase (ODC) and Proglucagon.

	c-jun	c-myc	c-fos	Proglucagon	ODC
Jejunum					
Control Vehicle	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0
Prednisone	0.97 ± 1.04	1.02 ± 0.99	N.S.	1.06 ± 0.13	0.93 ± 0.25
Budesonide	1.14 ± 0.55	1.05 ± 0.47	N.S.	0.64 ± 0.72	1.21 ± 0.26
Ileum					
Control Vehicle	1 ± 0	1 ± 0	N.S.	1 ± 0	1 ± 0
Prednisone	1.58 ± 0.67	1.40 ± 0.34	N.S.	1.40 ± 0.55	1.17 ± 0.17*#
Budesonide	1.16 ± 0.39	1.16 ± 0.32	N.S.	0.86 ± 0.45	0.96 ± 0.06

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.
N. S. = No signal.

*, $p<0.05$, prednisone vs control vehicle.
#, $p<0.05$, prednisone vs budesonide.

Table 9: Cytokine Gene Expression

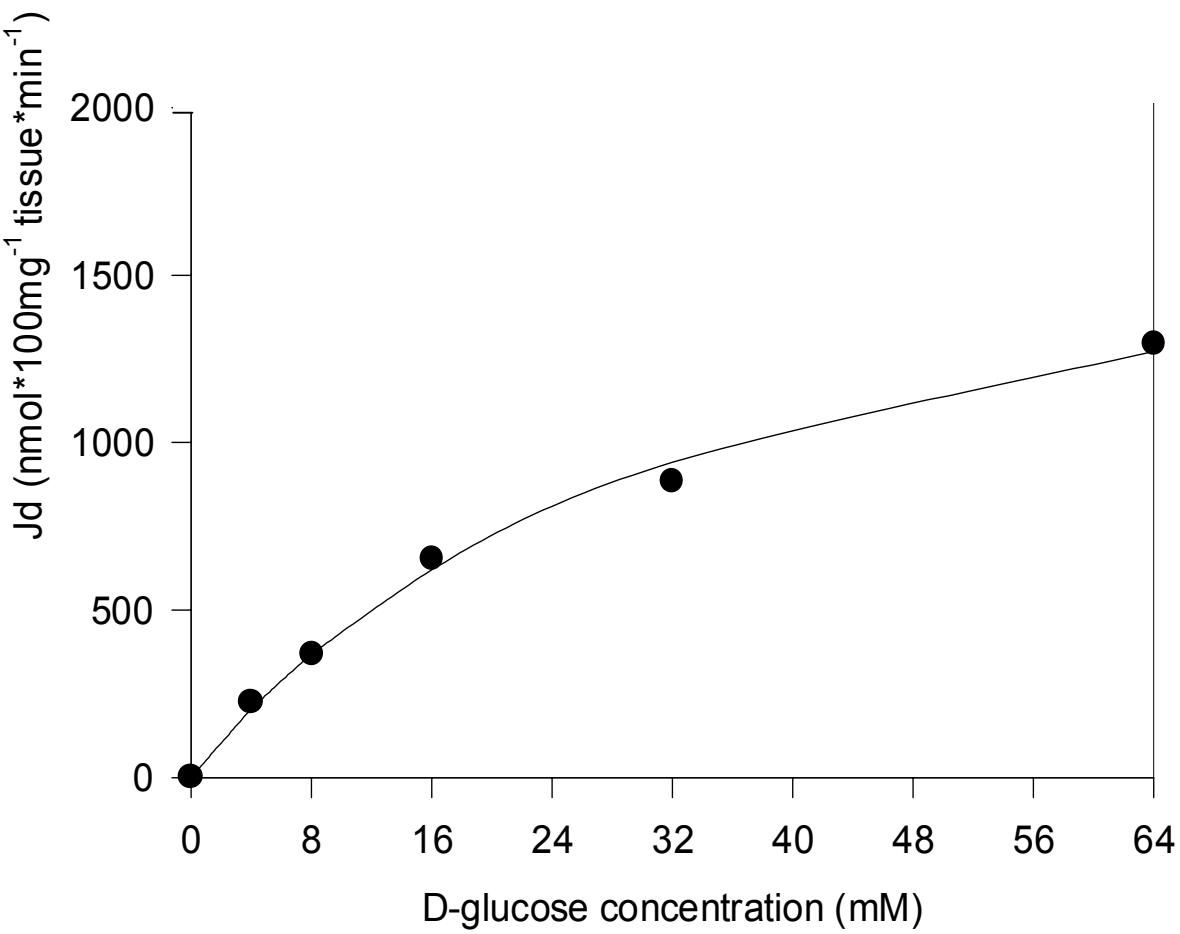
	TNF-α	IL-2	IL-6	IL-10
Jejunum				
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.32 ± 0.30	1.22 ± 0.55	1.23 ± 0.26	1.43 ± 0.40
Budesonide	1.41 ± 0.39	1.39 ± 0.43	1.25 ± 0.20	1.43 ± 0.36
Ileum				
Control Vehicle	1 ± 0	1 ± 0	1 ± 0	1 ± 0
Prednisone	1.42 ± 0.57	1.01 ± 0.19	1.20 ± 0.26	1.25 ± 0.33
Budesonide	1.25 ± 0.75	0.68 ± 0.17	1.02 ± 0.30	0.80 ± 0.11

Mean ± SD. The dose of budesonide was 0.25 mg/kg, and the dose of prednisone was 0.75 mg/kg.

Values were not significantly different.

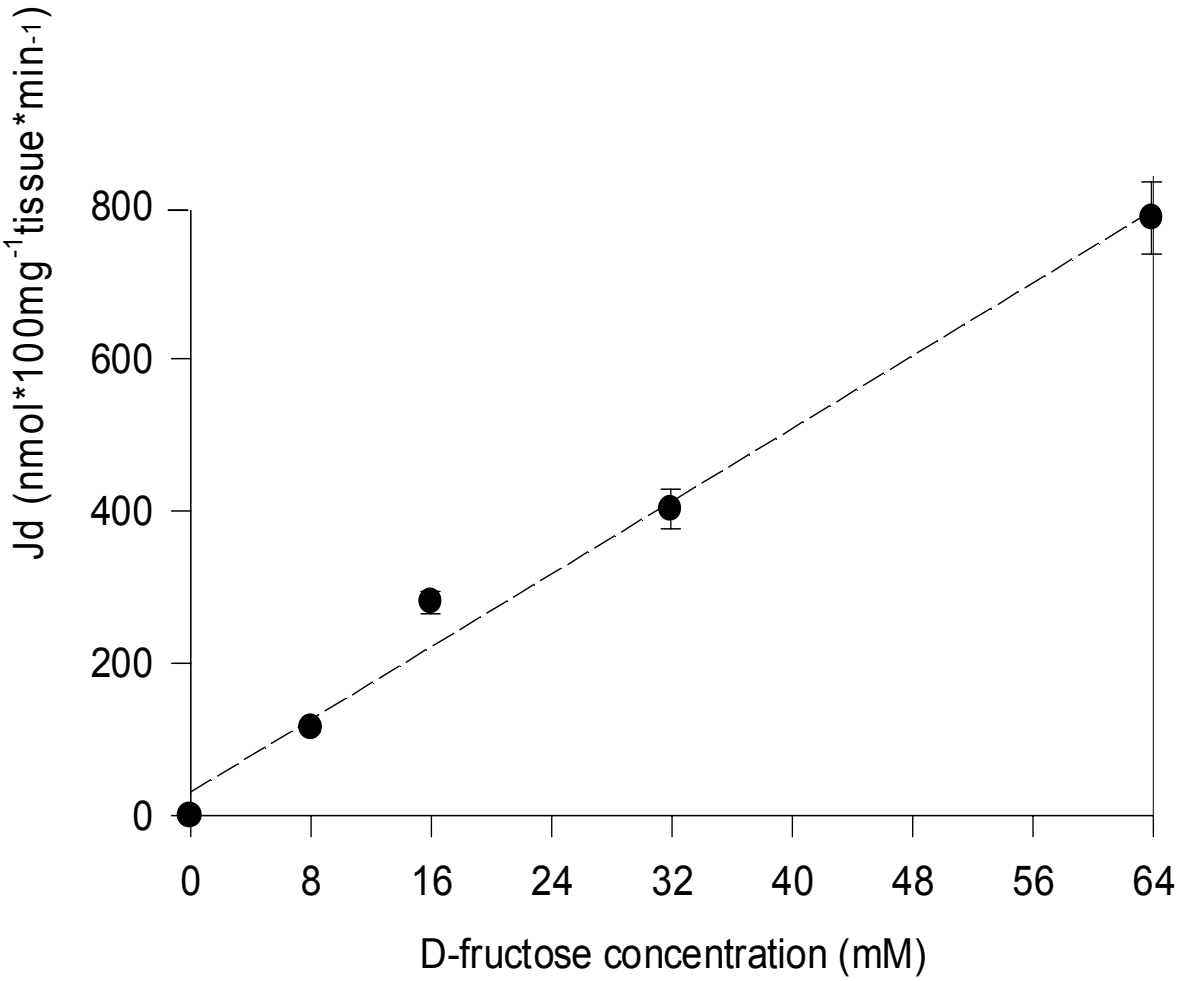
Figure 1: Relationship between Glucose Concentration and Jejunal Uptake

mg/kg



Budesonide was given as 0.74 mg/kg per day.

Figure 2: Relationship between Fructose Concentration and Ileal Uptake



Budesonide was given as 0.5 mg/kg.